The Australian Wine Research Institute Annual Report 2003

2003

Council Members

Mr R.E. Day, BAgSc, BAppSc Chairman
Elected a member under Clause 6(e) of the Articles of Association

Mr J.F. Brayne, BAppSc(Oen)
Elected a member under Clause 6(e) of the Articles of Association

Mr P.J. Dawson, BSc, BAppSc(Wine Science)
Elected a member under Clause 6(e) of the Articles of Association

Mr P.F. Hayes, BSc, BAppSc, MSc, DipEd
Elected a member under Clause 6(e) of the Articles of Association

Professor P.B. Høj, MSc, PhD
Ex officio under Clause 6(d) of the Articles of Association as Director of the Institute

Mr T.W.B. James, AssDip(WineProd)
Elected a member under Clause 6(e) of the Articles of Association

Mr G.R. Linton, BAppSc(AppChem), GradDip(SysAnal)
Elected a member under Clause 6(e) of the Articles of Association

Professor G.R. Scollary, MSc, PhD, BEd, BAppSc(Wine Science), FRACI
Charles Sturt University Representative under Clause 6(c) of the Articles of Association

Professor S.D. Tyerman, BSc(Hons), PhD
The University of Adelaide Representative under Clause 6(b) of the Articles of Association

Dr R.R. Walker, BAgSc(Hons) PhD
CSIRO Representative under Clause 6(a) of the Articles of Association

The Company

The Australian Wine Research Institute was incorporated under the South Australian Companies Act on 27 April 1955. It is a company limited by guarantee, it does not have a share capital and it has been permitted, under licence, to omit the word 'limited' from its registered name. The Constitution of The Australian Wine Research Institute sets out in broad terms the aims of the Institute and the Report of the Committee of Review for the Institute published in March 1977 identified the following specific aims:

1. To carry out applied research in the field of oenology.
2. To service the extension needs of the winemakers of Australia.
3. To be involved in the teaching of oenology at both undergraduate and postgraduate levels.
4. To assume responsibility for the co-ordination of oenological activities, and the collection, collation and dissemination of information on oenological and viticultural research to the benefit of the Australian wine industry.

The Institute's laboratories and offices are located within an internationally renown research cluster on the Waite Precinct at Urrbrae in the Adelaide foothills, on land leased from the University. The original lease is for a term of 99 years, with a right of renewal clause for a further 99 years. The Institute formally affiliated with The University of Adelaide in 1990. The first buildings were erected and opened in 1957 and alterations and extensions were completed in 1976. The buildings have been extensively modified and refurbished since that time with major extensions being undertaken in 1994 and 1999, and further expansion is planned.

The Institute is clustered with the following research and teaching organisations: Australian Wheat Management, BiometricsSA, three different Cooperative Research Centres (CRC), including the CRC for Viticulture, three divisions of CSIRO, Department of Water, Land and Biodiversity Conservation, Primary Industries and Resources South Australia (PIRSA), Provisor Pty Ltd, South Australian Research and Development Institute (SARDI) and The University of Adelaide's Faculty of Sciences (which includes the Schools of Agriculture and Wine and Earth and Environmental Sciences). Currently under construction to expand the research cluster is a new building to accommodate the Australian Centre for Plant Functional Genomics (APFG); Australian Genome Research Facility (AGFR); and Australian Grain Technologies (AGT).

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Acknowledgement

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49th Annual Report

Presented to
The Australian wine industry

30 June 2003

Chairman's report

The year that has just passed seems to have done so very quickly, but there is time to reflect on a number of events with significant portent for the future of The Australian Wine Research Institute.

Last year, when our Director Professor Peter Høj accepted the Maurice O'Shea Award for the AWRI he did so on behalf of 47 years of history at the forefront of wine research and on behalf of the combined efforts of several hundred past and present research staff and office holders. No doubt the founders of the Institute would have been pleased to see their vision recognised. It seemed also that this award underlined one of the intangible aspects of the Institute – its broader role in the wine industry. That the Institute has always had a minor and reluctant role in providing independent and objective commentary, when needed, is obvious enough if one cares to examine wine 'issues' of the past. Indeed, it is hardly surprising that this role should have evolved in an industry that uses technology as an important part of its positioning. Our involvement in providing the technical and research input to support the regulatory activities of the AWBC has been longstanding and essentially a behind-the-scenes activity. The current trend is for technical trade barriers to be invoked more frequently and with more vigour and emotion than in the past and, if this continues, the AWRI role in these areas is likely to become more important. We don't make policy at the AWRI, but the willingness and the expertise of our staff means that we are well placed to help those who do, with the goal of achieving a holistic success for our industry.

Early this year, we were very pleased to welcome Professor Isak (Sakkie) Pretorius to the position of Director of Research. Even for an incurable workaholic, the exercise of reading Sakkie's CV is a very revealing one and likely to result in feelings of having underachieved. Sakkie, who has quickly settled into life at AWRI, has strengthened an already vibrant team and we look forward to his contributions in the future. The Institute is very appreciative of the GWRDC Board's role in facilitating this important capture for the Australian wine industry.

One of the potentially most rewarding challenges faced by the Institute in the last year has been that of working together with its other shareholder partners in the genesis of Provisor (formerly NWIRC). Notwithstanding the potential challenges Provisor so clearly can create for existing service providers, the Institute was instrumental in its creation as it seemed to be an opportunity too good to miss for the wine industry. While the benefits of an additional entity in grape and wine research will be very clear as its operations gather momentum, the intangibles will also have significance for the way its shareholders work together in the future. Provisor will provide not only the bridging bricks and mortar between The Australian Wine Research Institute and CSIRO Plant Industry, but it will become the cultural cement between grape and wine research and strategic and programmatic research. The obligation for the AWRI, CSIRO Plant Industry, The University of Adelaide and SARDI to work more closely together will add to the critical mass of grape and wine research in Australia.

In today's world of multiskilling, cross disciplinary co-operations and virtual research institutions, the building of these types of links are not so much surprising but necessary, but they do place additional loads on the communication and administrative processes which support the science. The costs of these loads need to be recognized, factored in and appreciated as an investment in greater research productivity.

During the year, the AWRI made a submission to the Winemakers' Federation of Australia's R&D Review. The fact that the peak political body of our industry places sufficient importance on R&D to undertake a review of its direction and routes to its destination, speaks volumes for the ongoing role of R&D. Equally, this review process offers a valuable opportunity to improve communication of the benefits of R&D to the industry at large. It presents the opportunity to drive home the point that while, by many measures, we may pat ourselves on our collective back and claim great success, our spending on R&D is neither large nor assured.

In what will no doubt prove to be a very important assemblage of research work for the industry, the AWRI has, during the last year, made a start on a major research project into the area of *Brettanomyces* spoilage of wine. What *Brettanomyces* spoilage seems to lack in straight out destructive capability, compared to some other forms of spoilage, it more than makes up for this in terms of its insidious nature. As a spoilage at the near edge of the envelope, it produces a great deal of emotive response from ignorance and denial to preoccupation and paranoia. As a result, collecting the valid observations is difficult and the science shows every sign of being more and more complex as greater information is uncovered. The successful prosecuting of this little criminal critter will be a great challenge and will be indicative of a cohesive research and production community working together.

Some years ago, the work of our researchers on the key flavour compounds of oak cooperage led to techniques being developed to analyse the volatile phenolic constituents of wine, one of which (4-ethylphenol) was shown to be a key indicator of activity of *Brettanomyces*. This analysis has now become one of the most frequently demanded of our Analytical Services team. In a broadly similar way, a secondary output from a key research project again seems highly likely to provide an extremely useful tool for industry in a completely unrelated application. Demonstrations that juice protein profiles can be successfully used to fingerprint different varieties have resulted in numerous requests from industry to conduct juice fingerprinting analyses to aid in issues of varietal integrity. Currently, these techniques are being tuned so that they can be offered as routine analyses by Analytical Services. These are prime examples of the value of strategic research producing very valuable spin-offs which could never be planned, budgeted or anticipated.

In recent months, the process of examining existing resources in conjunction with our Provisor partners has highlighted the limitations imposed on our researchers by the physical constraints of our existing buildings. The rapid growth in R&D output from highly focussed medium term projects being funded from a very competitive funding environment, has invariably meant that we are, in wine R&D, on 'a beer budget.' The rhetorical question is: How do we identify the wine R&D philanthropists who will catalyse provision of the kind of Institute worthy of a $4 billion industry and soon to be demanded by it?

Last year I mentioned the unflagging energy and task orientation of the Director, Professor Peter Høj, as deserving of special mention. During the last year, in very difficult personal circumstances, Peter has made it even more difficult to choose worthy descriptors to record his efforts. I would also like to acknowledge the efforts of all staff who have been so supportive both on a personal and professional basis.

Many Institute staff members again put a great deal of effort into extending the message into the industry via Roadshows, seminar and conference presentations and workshops. It is particularly gratifying to see staff members blossom into excellent presenters, a process which is reflective of the pride and professionalism applied to their primary research tasks.

Finally, I would like to thank my fellow Councillors for their energy and thoughtful insights in helping to shape the strategic direction that we hope will enable us to meet best the next set of challenges.

Robin Day
Chairman

Robin Day - Chairman

Council notes

Chairman
At the Council meeting held on 29 October 2002, Mr R.E. Day was elected Chairman of Council.

Members of the Executive Committee
Mr R.E. Day
Professor P.B. Høj
Professor S.D. Tyerman
Mr T.W.B. James
Mr G.R. Linton

Deputy Members of Council
Mr N.P. Blieschke
Mr L.P. Deans
Dr P.R. Dry
Mr A.M. Kennedy
Mr D.J. McWilliam
Mr J. Northey
Dr. N.S. Scott

Audit Sub-Committee
Mr R.E. Day
Mr P.J. Dawson
Mr T.W.B. James

Meetings
Ordinary General Meeting
The 48th Ordinary (Annual) General Meeting was held on 29 October 2002.

Council
The Council of the Institute met on the following dates: 23 July 2002, 24 July 2002, 29 October 2002, 25 February 2003, 5 May 2003. Executive members of Council met on 3 December 2002.

Funding
The Council of the Institute acknowledges the continuing financial support of the Grape and Wine Research and Development Corporation.

Appreciation
The Institute acknowledges the assistance and cooperation of the following organizations throughout the year:

Australian Wine and Brandy Corporation
Charles Sturt University
Commonwealth Scientific and Industrial Research Organization (CSIRO)
Cooperative Research Centre for Viticulture
Department of Agriculture, Fisheries and Forestry Australia
South Australian Wine and Brandy Industry Association Inc.
State Departments of Agriculture
State Government of South Australia
The University of Adelaide
Winegrape Growers' Council of Australia, Inc.
Winemakers' Federation of Australia Inc.

Director's report

Solving problems is no longer sufficient; defining and unleashing hidden potential is a new competitive imperative

The Australian grape and wine industry is a complex mix of different sized companies who collectively export $2.4B worth of wine per annum and who share a vision of what world wide consumers expect and will continue to demand. The industry invests in R&D and has reaped benefits from adopting new technologies and processes to keep testing and challenging the world stage.

The changing focus of grape and wine research: In past decades, much grape and wine research has focused on identifying and managing problems in the vineyard and winery. Research has played a key role in underpinning industry growth, by reducing risks such as disease in the vineyard; microbially induced faults; and suboptimal closure performance. Parallel aspects of research and development have related to reducing labour inputs through mechanisation in both vineyards and wineries to deliver quality products at competitive price points. Significant challenges still remain in dealing with issues such as smarter application of irrigation water; further reduction of unintended microbial activity; better ways to close a bottle to ensure durable capture of quality; and to reduce production costs further. Nevertheless, there is also a desire to stretch the boundaries to improve wine. Tailored production inputs can unleash hidden potential that will enhance product attributes and value.

The holy grail and its elusive nature: One of the grandest R&D aspirations of the wine industry is to develop objective measures for grape and wine quality, a feat that despite some considerable progress still remains elusive. Not only is the composition of wine challenging to unravel, but changing consumer and market preferences will dictate the type of measurement that will be important. For example, in the 1960s Australian domestic production comprised in excess of 75% fortified wine and it was not until the early 1970s that Chardonnay was first introduced by Murray Tyrrell to Australia. In contrast, fortified wine now accounts for less than 5% of Australian wine production while Chardonnay comprises almost 20% of total wine production. Readers of the many informative 'wine columns' now available will also be aware that in addition to consumer preferences changing over time, the ranking of quality attributes also varies amongst individuals. The research challenge is to set clear goals from these variable messages, so that management options for the grapegrowers and winemakers can be tested.

The nature of the technical challenge: Wine has been drunk for millennia but it is only in the last sixty years we have understood the universal biochemical pathway for fermenting large quantities of sugar to alcohol. Today, wine researchers and innovators are grappling with how to identify, measure and manipulate compounds which, when present in levels as low as 0.000.000.001 grams per litre, significantly change the sensorial attributes of the wine. Passion-fruit character in white wines is contributed by 3MHA (3-mercaptohexyl acetate) at such low concentrations. This aroma compound is exceedingly potent, for example, one gram of 3MHA would be, in principle, enough to impart a passion-fruit character in a quantity of liquid equivalent to the size of 100 Olympic-sized swimming pools. Similar potency can be ascribed to the compounds giving rise to the 'green' and herbaceous capsicum character in white wines, which can be attributed to isobutyl methoxypyrazines (IBMP). The current challenge and focus for wine and grape researchers is to be able to accurately control the composition of wine with respect to potent impact compounds such as 3MHA and IBMP through inputs across the whole production chain from vineyard to consumer palate. The challenge can be illustrated with reference to these two compounds in the following hypothetical.

Peter Høj accepting the 2002 McWilliam's Wines Maurice O'Shea Award on behalf of The Australian Wine Research Institute

An illustration of the approach to current research: Assume an extensive marketing survey of consumers has concluded that there is a niche for a white wine which has a strong hint of tropical fruit characters (including passion-fruit) with only a moderate herbaceous capsicum component. Assume also that extensive research had shown that of the 800 or so known volatile compounds in wine, the two target compounds 3MHA and IBMP alone accounted for the tropical and capsicum characters of wine and that all consumers are equally sensitive to the two classes of compounds (both assumptions represent a gross oversimplification, the reality is much more complicated). In order to assist the Australian wine industry to make wines that fit the target niche market, the following research activities (which are illustrative of much research taking place today) could be envisaged:

1. Establish the target concentrations of 3MHA and IBMP that give rise to the desired sensorial property of the wine. Such research involves repeated correlations of sensorial assessment and analytical chemistry of preferred wines.

2. Establish the origin of the target compounds. Are they derived directly from grapes or formed during winemaking? As it turns out, IBMP is derived directly from grapes but 3MHA is not. The latter is formed from a precursor in grapes during microbial fermentation. Researchers would thus typically have to identify the precursor in grapes.

3. Assuming now that the origins of the target compounds are fully understood, researchers will have to establish which vineyard practices influence the abundance of the target and precursor compounds in the grapes. For example, in the case of IBMP, it is now firmly established that exposure of grapes to sunlight diminishes the concentration of this compound. Accordingly, if a low capsicum character is required, viticultural practices would be implemented to ensure good to moderate bunch exposure of the grapes. A second level of complexity is introduced at this stage, namely that of vineyard variability. Using the tools of precision agriculture, ongoing research has clearly established that considerable variability with respect to vine performance exists in many vineyards. An important aspect of ongoing research is thus to define and manage variability to pick like fruit with like fruit in order to achieve the target compositions better with respect to key impact compounds such as 3MHA and IBMP.

4. Once the grapes are at the winery, choices about processing will have to be made. For example, are some of the target

components present in the skin and others in the juice? Judicious choice of juice/skin contact time is then required as longer skin contact will increase the relative proportion of the skin component relative to the juice component. Further, of the plethora of yeasts available to winemakers – will some yeast influence the final concentration of the target compounds? In the case of the IBMP this is probably not so, however in the case of 3MHA, the efficiency of its formation from the grape-derived precursor is very dependent on yeast strains and the correct choice of yeast strain is, therefore, a major determinant of whether the target for the passion-fruit aroma imparting compound is hit.

5. Now to bottling of the finished product. What closure to choose? When would we expect the product to hit the market, be sold and consumed? These considerations are crucial as our target compounds have differing stability in wine. It would be safe to assume that, in this hypothetical example, the stability of the two target compounds differ and that the optimal relative concentration of 3MHA and IBMP is only achieved through a relatively short time span of the wine's life in bottle. The key here is to understand the relative speed at which the concentration of the target compounds change. Combined with the knowledge that more than 90% of wine is likely to be consumed within one year of release, the target concentrations of 3MHA and IBMP can thus be set.

Sounds simple enough, but needless to say that this is a very challenging task and winemaking will, therefore, remain a wonderful combination of art, experience and science for many decades to come. Let us remember that the task outlined above will involve many research groups, field and laboratory work, close interactions with growers and winemakers, good design, and some good luck for the whole process to work. It may take millions of dollars of investment and several vintages to deliver the outcome. It also reminds us of what the industry expects of its R&D: an outcome that continues to provide competitive market advantage for the Australian industry.

The approach required is clearly multi-disciplinary and will require enhanced collaboration within institutions and between institutions. It relies on an increasingly sophisticated and well-educated workforce that is able to apply relevant information to achieve outcomes specified as desirable by industry. As always, high impact outcomes rely on basic strategic research to unleash new knowledge; its testing and examination under applied conditions; and an industry that will join in the process of translating all of this information into a commercial activity. The Australian industry is characterised by individuals that have seen the success from such innovative/questioning activity – a fact that is catalytic and encourages further gains in the daily approach to improved production.

A second characteristic of the Australian wine industry is that the basic research is informed and prioritised by practical goals and observations.

Finally a word on the expenditure necessary to reach these goals. The majority of grape and wine R&D in Australia is financed by the Grape and Wine Research and Development Corporation (GWRDC), a partnership based on industry and Commonwealth Government co-investment. Through the GWRDC, the industry contributes about $8M p.a. to R&D – is the industry receiving value for money? As the Australian winemakers repeatedly seem prepared to increase their levy contributions, the general sentiment is almost certainly yes. As outlined in a previous Annual Report, most people would agree that R&D over the past 30 years or so would have improved the bottom line per bottle of wine produced by at least 10 cents through genuine improvement in quality, reduced labour inputs etc. This translates into a return of about $150-160M p.a. across the industry, not a bad return on investment - just ask producers and tax-payers in Europe. There, approximately $2B of subsidies are required to keep a traditional non-innovative industry afloat while watching the 200-fold growth in Australian wine exports over the past two decades. This is indeed an impressive achievement but one thing is for sure, more R&D investment is needed to maintain the position as a leader in such a competitive global industry and it is to be hoped that the wine industry will enhance that investment in a relatively fast manner through a collective levy increase as suggested by the Winemakers' Federation of Australia.

The Australian Wine Research Institute has a proud 48 year history of serving the wine industry with strategic R&D and it is, therefore, not surprising that the Grape and Wine Research and Development Corporation, on a competitive basis, awards approximately 38% of its total R&D budget to the Institute, a contribution we are most grateful for. This is not only to help the Australian wine industry to address and avoid problems but also, as illustrated above, to help unleash hidden potential in our grape material. This phase of R&D activity is likely to be even more challenging than past research efforts and the Institute, with its rich history of achievement and strengthened focus on cross-disciplinary research, is in a prime position to take on this challenge. We are looking forward to serving the industry in this capacity and believe we have the credentials to do so; a belief underscored by events of 2002/2003:

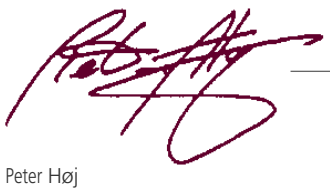
> Industry itself has recognised our contribution by awarding the prestigious **Maurice O'Shea Award** to the Institute in August 2002. This constitutes a recognition of the value industry places on the past and present staff activities and its direct importance for the Australian wine industry's bottom line.

> The Institute has a unique collection of highly professional staff that is totally committed to serving its industry stakeholders. This represents a truly national capacity that would take decades to assemble from scratch and without which our industry will be much harder pressed to counter the inevitable competitive efforts from other wine producing countries. In 2003, we further strengthened our capacity by attracting Professor Isak Pretorius to the Institute in the role as Director of Research. **Professor Isak Pretorius** was the Foundation Director of the Institute for Wine Biotechnology in Stellenbosch and is recognised as one of the world's leading wine microbiologists.

> Yet again, many **research highlights** with direct commercial relevance are reported elsewhere in this report. These include the important insights in respect of **'flavour scalping'** by closures, discovery and characterisation of stable red wine pigments as well as new **flavour compounds**, better understanding of factors affecting **tannin astringency**, commercial application of **new yeast strains**, early signs that Institute support and development can reduce unwanted spoilage of wine by **Brettanomyces** etc.

> Continued and increasing support for our commercial Analytical Service which now conducts in excess of **54,000** analyses per annum and hence constitutes a major portal for technology diffusion to industry.

Past, present and future achievements in Institute R&D continue to depend on a strong partnership with industry, R&D investors and Institute staff. This year, a special thanks goes to Dr Peter Costello who, after more than a seven years service has taken up a role as National Technical Manager with Lallemand Australia and also to Mr Don Buick who elected to retire after having made a tremendous contribution to our Analytical Service which has almost doubled its turn-over since 1997.



Peter Høj
Managing Director

Staff

Peter Bordier Høj, MSc, PhD, *UCopenhagen*, Managing Director
Isak Stephanus Pretorius, BSc(Agric)(Hons), MSc(Agric), PhD, *Orange Free State*, Director of Research

Research team members

Paul Anthony Henschke, BSc(Hons), PhD *UAdel.,* Principal Research Microbiologist
Mark Aidan Sefton, BSc(Hons), PhD *UWA,* Principal Research Chemist
Elizabeth Joy Waters, BSc, PhD *UAdel.,* Principal Research Biochemist
Robert George Dambergs, BSc(Hons) *UAdel.,* PhD *UQld,* Senior Research Chemist
Miguel Antonio de Barros Lopes, BSc *Oregon*, PhD *UC (Santa Barbara),* Senior Molecular Biologist
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Markus Johannes Herderich, PhD *UWuerzburg,* Senior Research Chemist
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Computer Systems Officer
George Kyriakos Skouroumounis, BSc(Hons) *Flinders,* PhD, GradDipOenol. *UAdel.,* Research Chemist
Paul Alexander Smith, BSc(Hons), PhD *Flinders,* Research Chemist (commenced 5 May 2003)
Patrik Raymond Jones, BAgSc, PhD *UAdel.,* Research Chemist (commenced 14 April 2003)
Paul Joseph Chambers, BSc(Hons), PhD *Hertfordshire* (commenced 6 January 2003), Visiting Research Fellow
Diego Torrea, BSc, PhD, *Universidad Publica de Navarra* (commenced 30 September 2002), Visiting Postdoctoral Research Fellow
Stéphane Vidal, Eng Dip. (Biochem), *Nat. Instit. App. Sc. Lyon,* MBiochem, *UClaude Bernard Lyon,* PhD *UJoseph Fourier Grenoble,* Postdoctoral Research Fellow (concluded 22 November 2002)
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Kate Alexandra Lattey, BSc, *Canterbury,* Chemist/Sensory Analyst
Tangerine 'Mango' Parker, BSc, *Flinders,* Chemist
Tracey Ellen Siebert, BSc *UAdel.,* Chemist
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Senior Computer Systems Officer
Lorelie Flood, BFTech *Xavier,* BSc *UNew England,* Research Assistant (commenced 20 November 2002)
Leslie Joseph Janik, AssDipIndChem *USthAust.,* MAppSc *USthAust.,* Technical Research Officer
Simon Justin Dillon, BSc(Hons) *Flinders,* Research Assistant
Shauna Liam Brown, BBiotech(Hons) *Flinders,* Research Assistant (from 28 April – 30 June 2003)
Gayle Ann Baldock, BSc(Hons) *Guelph,* Technical Officer/Casual Analyst
Wieslawa Cynkar, BSc, PhD *Wroclaw,* Technical Officer
Maria Jolanta Kwiatkowski, MSc *Gliwice,* Technical Officer
Holger Gockowiak, BSc(Hons) *UAdel.,* Part time Laboratory Manager
Kevin Herbert Pardon, AssDip(AppChem) *SAIT,* Laboratory Technician
Jennifer Rose Bellon, Technical Assistant
Jane Melissa McCarthy, AdCertMedLabSc *USthAust.,* CertVetNurs, CertAnimHand *TAFE,* Technical Assistant
Merran Alida Daniel, BTech, BSc(Hons) *Flinders,* Postgraduate Student
Maria Josephine de Sa, MSc *Nottingham,* BSc(Hons) *Brunel,* Postgraduate Student
Jennifer Gardner, BSc *UAdel.,* Postgraduate Student
Antonio Grimaldi, MSc (equiv.) *Florence,* Postgraduate Student
Christophe Guirado, MSc *ENSBANA,* Visiting French Postgraduate Student (until 25 October 2002)
Anthony John Heinrich, BBiotech(Hons) *Flinders,* Postgraduate Student
Kate Susan Howell, BSc(Hons) *UNSW,* Postgraduate Student
Agnieszka Janusz, BSc(Hons) *Flinders*, Postgraduate Student
Oenone Jean Macintyre, BSc, BE(Chem)(Hons), *UAdel.,* Postgraduate Student
Richard Anthony Muhlack, BE(Chem)(Hons), *UAdel.,* Postgraduate Student
Carolyn Jane Puglisi, BSc *Flinders,* BSc(Hons) *UAdel.,* Postgraduate Student
Heather Eunice Smyth, BSc(Hons) *UAdel.,* Postgraduate Student
Kerry Leigh Wilkinson, BSc(Hons) *Flinders,* Postgraduate Student
Rachel Christine Brown, BTech(For's & AnalytChem), *Flinders,* Honours Student
Matthew Carlyle Caldersmith, BSc(Hons) *UAdel.,* Honours Student
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Jaromir Guzinsky, BBiotech, *UAdel.,* Honours Student
Nicola Renee Sleep, BTech(For's & AnalytChem), *Flinders,* Honours Student

See Appendix 3 for details of all students supervised by Institute staff

Industry Services team members*

Peter William Godden, BAppSc(Wine Science) *UAdel.,* Winemaker/Manager Industry Services
Mark Gishen, BE(Chem)(Hons), MEngSc(Chem) *UMelb.,* Quality Liaison Manager
Sally-Jean Bell, BSc(Hons) *UWA,* GradDip(Wine) *Roseworthy,* PhD *UWA,* Viticulturist
Adrian Dermott Coulter, BSc *Flinders,* GradDipOenol, *UAdel.,* Oenologist
Geoffrey David Cowey, BSc(Hons) *UAdel.,* Chemist (commenced 4 November 2002)
Ella Margaret Clare Robinson, BA, BSc(Hons) *UAdel.,* Chemist
Raelene Joan Blair, CertAppMgt(Marketing) *AIM,* Communication and Publicity Manager/Personal Assistant to the Director/Conference Manager
Creina Standish Stockley, BSc(Hons) *UAdel.,* MSc *Flinders,* MBA *USthAust.,* Health and Regulatory Information Manager
Catherine Grace Daniel, BA *ANU*, GradDip(Lib) RMIT, Librarian
Ingrid Betty-Maud Oats, DipLibInfo *Adel. Tafe,* Library Technician
Melissa Elizabeth Francis, BA *UMelb*., DipEd *Melb. State Col.* Library Assistant

*Several members of the Industry Services Team lead or take part in a number of research projects.

Administration

Hans Engelbert Muhlack, BEc *UAdel.,* CPA Aust., Company Secretary
Rachel Lee Edwards, Accountant
Heather Margaret Donnell, Secretary to the Director
Rhonda Irene Packer, Administration Officer (commenced 2 October 2002)
Pauline Jorgansen, Administration Support (commenced 10 March 2003)
Julie McConnell, Administration Support (commenced 18 March 2003)
Emma-Kate White, Receptionist
Jelena Jovanovic, Laboratory Assistant Function Support

Analytical Service

Donald Robert Buick, BSc *UAdel.,* GradDip (BusAdmin) *USthAust.,* AIFST, Manager – Analytical Service (retiring 1 July 2003)
Peter Charles Hans Eichinger, BSc(Hons), PhD *UAdel.,* Manager – Analytical Service (commenced 2 June 2003)
Sandra Margaret Lloyd-Davies, BA *Flinders,* Customer Service Officer
Matthew Grant Holdstock, BSc *Flinders*, GradDipOenol *UAdel.,* Analytical Service Supervisor – Laboratory
John Benjamin Hughes, DipWineMrktg *UAdel.,* Analytical Service Supervisor – Administration (concluded 13 September 2002)
Gregory Andrew Ruediger, BAppSc *SAIT,* GradDipOenol *UAdel.,* Trace Analysis Laboratory Supervisor
Randell Leith Taylor, BSc(Hons) *UAdel.,* Chemist
David Rolfe Boehm, BSc *UAdel.,* Technical Officer
Amanda Louise Cook, AdvCert (Lab Tech) *Mackay*, Senior Laboratory Technician
Belinda Bramley, Casual Laboratory Technician
Heather Mandy Brooks, Casual Laboratory Technician
Anna Catalano, Casual Laboratory Technician
Matthew James Cream, Casual Laboratory Technician
Danielle Kylie Leedham, Casual Laboratory Technician
Athina Massis, DipAppSc, *USthAust.,* Casual Laboratory Technician
Maria Concettina Mills, Analytical Service Administration Support

The Institute was awarded the prestigious *McWilliam's Wines Maurice O'Shea Award* in recognition of its contribution to the success of the Australian wine industry over the past 48 years.

The Institute attracted one of the world's very best wine microbiologists, Professor Isak Pretorius, to the position of Director of Research with effect from January 2003. This augurs well for the future of the Institute and it is a reflection of the high esteem in which it is held. The strategic foresight of the GWRDC in assisting this critical development is acknowledged gratefully.

Using organic synthesis we proved the structures of two malvidin-derived wine pigments and established their colour properties. With the help of these synthesised reference compounds we demonstrated that the pigments, which we had detected previously for the first time in red wine, were almost inert to SO_2-mediated bleaching, and that they showed superior colour properties and enhanced extinction coefficients in model wine at pH 3.6 when compared to grape-skin anthocyanins.

A method for the measurement of acetaldehyde has been developed and validated. This enables us to monitor acetaldehyde concentrations in various tannin-, yeast- and wine oxidation-related research projects and will assist in developing a more detailed picture of this reactive aldehyde.

With the help of rapid HPLC methods developed by the Tannin team, more than one thousand samples from research projects of the Tannin, NIR and Microbiology teams have been analysed to establish anthocyanin, tannin and 'pigmented polymer' concentrations in samples from viticultural and winemaking trials. These data are vital to improve our understanding of the correlation between grape polyphenols, and the composition, colour and mouth-feel of wine. They also form the basis for our investigations into the potential of NIRS for the efficient measurement of polyphenols.

Using solvent step-gradients with multilayer coil countercurrent chromatography (MLCCC), the isolation of anthocyanins and coumaroylated anthocyanins from commercially available grape marc extracts was optimised. In addition, MLCCC was instrumental in enriching very polar and potentially novel pigments for structure elucidation efforts, and in isolating 'pigmented polymers' for sensory studies.

2,3,6-Trimethylphenylbutadiene (TPB) has been identified as a hitherto unrecognised wine flavour compound that is formed by hydrolysis of grape-derived glycoconjugates. The threshold of TPB in a white wine was determined to be 40 ng/L and sensorial descriptors ranged from 'floral', 'geranium' and 'tobacco' at the lower concentrations to 'pungent', 'very green', 'unpleasant', 'plastic', and 'insecticide' at concentrations of 270 ng/L and above. This makes it one of the most potent wine flavour compounds known. Identification of TPB in wines has furthered our understanding of the contribution of grape secondary metabolites to wine quality.

Four isomeric potential damascenone precursors, have been synthesised. The availability of these authentic samples allowed us to prove that they were intermediates in the formation of the important wine aroma compound damascenone. These findings have led us to re-evaluate the mechanism by which damascenone is formed in wine and other food products and will facilitate our goal of being able to improve wine quality by manipulation of wine composition in the vineyard and in the winery.

Glucosides of the open-chain forms of *cis*- and *trans*-oak lactone have been synthesised. These compounds are stable in solution at wine pH, but are converted to the corresponding lactones at barrel-toasting temperatures. This demonstrates that such compounds can act as precursors of oak lactone during barrel manufacture, but not during subsequent wine conservation. By separating the isomeric glucosides from each other we have also been able to resolve the nature-identical isomer of *cis*-oak lactone from its non-natural optical isomer and evaluate its sensory properties. Previous sensory work on *cis*-oak lactone in wine has been carried out on mixtures of these two forms (i.e. the racemate), or even on mixtures of *cis*- and *trans*-oak lactone. The sensory detection threshold of the nature-identical isomer of *cis*-oak lactone (which is ten times more potent than the *trans* form) in a neutral dry white wine was 23 mg/L. This is much lower than the value of 90 mg/L previously reported for the racemic mixture.

We have demonstrated that at wine pH, (3.0 – 3.8), oak lactones exist almost entirely in the aroma-active lactone form at equilibrium, in contrast to an earlier hypothesis. At the highest pHs studied, however, the open chain form of the *cis*-isomer lactonised relatively slowly, indicating the need for suitable soaking times and low pHs of wine or aqueous extracts when preparing extracts of oak shavings for analysis. The data show that wines treated with oak chips or shavings over a few days could continue to develop 'oakiness' by additional formation of *cis*-oak lactone once the chips or shavings are removed.

Sensory studies robustly established that purified anthocyanin glucosides and their corresponding coumarates were not perceived as bitter or astringent. In addition, these grape pigments did not influence the fullness or viscosity of the solutions in which they were tasted.

The effect of ascorbic acid addition on oxidation of wine continues to be a matter for debate in the literature. Previous laboratory scale work from the Institute (Institute publication #577) and others indicated an enhanced depletion of sulfur dioxide in wine and model solutions to which ascorbic acid was added but formal sensory assessment has rarely been conducted in these experiments. Through collaboration with a winery we have now examined the effect of ascorbic acid addition to a Riesling and a Chardonnay wine bottled on a large scale under commercial conditions. Aroma, colour and compositional data indicate that for four closure types examined, these two wines were less oxidised after storage under ideal conditions for two and a half to three years when ascorbic acid was added at bottling. When analysed after six months' storage there was little difference between the wines (Institute Annual Report 2000).

The potential for mass spectrometry of red and white juice proteins to differentiate varieties of *Vitis vinifera* has been further confirmed. The extent to which the discriminating power can be extended to white wine is limited if bentonite fining has occurred. However, as DNA typing appears not to be practically possible for wine samples, the extension of our findings might represent one of very few avenues by which varietal authentication of white wine can be executed with some degree of certainty, if necessary through a combination of analyses of secondary metabolites and, of course, sensory analyses for varietal typicity.

Milligram quantities of a haze-protective factor (6xhis Hpf2p) have been overexpressed in yeast and purified. This has allowed us to treat a wine and assess its short to medium term stability during storage at 25°C. After three and six months' storage, 6xhis Hpf2p had retained full haze-protective activity, an important prerequisite for commercial viability in relation to the prevention of protein hazes in white wine.

Methods to measure some volatile thiols of importance to varietal flavour have been developed and are now being applied to identify commercial wine strains that release either high or low amounts of these important aroma compounds. These findings will initially be important for modifying the flavour of a wide range of varietal wines.

A collaboration with The Australian Proteomic Analysis Facility (APAF) in Sydney has permitted the identification of proteins that are differentially expressed in wine yeast strains. In the longer term, it is hoped that this research will identify factors that provide wine yeast with their important winemaking properties and is expected to lead to the generation of improved strains.

Flavour metabolites that are produced at much higher concentrations in *Saccharomyces* species that are generally not associated with winemaking have been identified. These results indicate that interspecific hybrid yeast offer an effective strategy to harness diverse flavours in wine.

Winery trials made in various wine regions with AWRI isolates of *S. bayanus* yeast are confirming our laboratory trials that have shown that these yeast can impart a greater diversity of sensory characteristics than achieved with *S. cerevisiae,* especially to aroma and palate fullness and texture. Demand for scaled-up winery trials with these *S. bayanus* yeast has increased to the point that we have engaged Lallemand to develop commercial starter cultures.

A random amplified polymorphic DNA (RAPD) method for determining genetic similarity and differentiating strains of commercial and wine isolates of the malolactic bacterium *Oenococcus oeni* has shown that: (i) all commercial strains tested are genetically distinct; (ii) Australian isolates from different wineries are genetically distinct and at least as genetically diverse as the commercial strains; and (iii) isolates from a given winery may be genetically diverse.

The adventitious growth of spoilage acetic acid bacteria in bottled red wine stored in an upright vertical, but not horizontal position, has led us to hypothesise that the upright storage position created a heterogeneous environment that allowed the growth of a

bacterial film in only those bottles sealed with cork closures with substantial permeability to oxygen. Such a heterogeneous environment would likely not exist in horizontally stored bottles since the larger volume of wine adjacent to the cork would strongly compete with the bacteria for the oxygen as it diffuses through the cork closure. The direct measurement of oxygen content of spoiled and non-spoiled wines support this hypothesis which has been published in an international scientific journal (Institute publication #718), but still requires further rigorous confirmation.

The Industry Services' team responded to 1,676 enquiries during the year, and analysed 2,231 samples (an 81% increase in sample analysis over the previous year).

A record number of Roadshows were conducted, with ten seminar days and five workshops being held in four states.

The flow of new information from the Institute's closure trial continued, with three publications during the year (two in *Technical Review* and one conference paper [see Appendix 1]). Nineteen interviews were conducted with media and trial results were used or referred to in a number of other articles published both in Australia and overseas.

The Institute's Dekkera/Brettanomyces control seminar was presented on 19 occasions during the year to approximately 530 winemakers, and has been presented to approximately 1,550 winemakers since 1999. An ongoing targeted survey of the composition of Cabernet Sauvignon wines from five Australian wine regions indicates an apparent fall in the concentration of key *Dekkera/Brettanomyces* yeast derived spoilage compounds in wines from the 2001 vintage, compared to the previous five vintages. The basis and durability of this pleasing trend is to be investigated further.

A trial conducted in conjunction with grapegrower and winemaker associations and wine companies into the effects of bushfire smoke on grapes and wines, elucidated an understanding of the nature of the problem and possible taint-minimisation strategies.

Efforts to develop a practical NIR method for determining colour, total soluble solids (TSS) and pH in red grapes continue and have been outlined in an industry publication (Institute publication #730). During the 2003 vintage, the total number of samples analysed by NIRS represented approximately 300,000 tonnes of fruit, in excess of 20% of the total national harvest. Significant benefits have also been demonstrated from the use of NIR in the research environment. Using NIR to predict red grape quality parameters (colour, TSS and pH), researchers at CSIRO (Land & Water's Precision Agriculture group) and The University of Adelaide (Roseworthy campus) have been able to significantly reduce the cost of analysis and the necessity for wet chemistry in relation to their research projects.

Preliminary results have indicated that neither freezing of red grape samples prior to homogenisation, nor the type of homogeniser had any significant effect on the accuracy of laboratory determinations of colour and TSS. However, as one might expect, there was an effect of freezing on pH. Work is continuing with further samples remaining in frozen storage for later analysis to investigate any long-term stability effects.

The Institute has been involved in the testing and assisting in the development of an automated grape sample preparation system 'Bioprep5 Robotics', which has been released commercially by a local Adelaide company. The results from samples prepared with the automated system compared well to those obtained by the manual method in terms of accuracy and precision. The unit appears to offer potential in speeding up the preparation of red grape homogenates for scanning by NIR (up to 112 samples per 8 hour day) when configured to act as an automated homogeniser.

An evaluation of the feasibility of NIR spectroscopy for detection of *Botrytis* and powdery mildew in grapes is continuing in collaboration with the Fungal Pathology Laboratory of The Adelaide University and CSIRO Plant Industry in Canberra. Distinct spectral changes were observed in fruit that had been visually classified into a number of different levels of powdery mildew infection. Principal component analysis of the spectra was used to detect and correctly classify, powdery mildew infection down to at least a 10% infection level. Similar work is also in progress with *Botrytis*-infected fruit. The implication of this work is that it may be possible to discriminate fungal infected fruit at the weighbridge to provide a 'go/no-go' test to highlight suspect fruit for further detailed analysis to determine suitability for winemaking.

Stable isotope dilution analysis methods for 33 important fermentation esters, acids and alcohols using their deuterium labelled analogues as internal standards have been fully validated. The methods have facilitated accurate and rapid measurement of the composition of wines and model media ferments and are are now being applied to a range of cross-disciplinary projects at the Institute including that of flavour scalping by closures.

Stable isotope dilution analysis methods for wine taint compounds derived from petroleum products, including toluene, styrene, C2 alkyl benzenes (e.g. xylenes), C3 alkyl benzenes (e.g. trimethylbenzenes), C4 alkyl benzenes (e.g. tetramethylbenzenes), naphthalene, methylnaphthalenes, dimethylnaphthalenes and trimethylnaphthalenes have been fully validated. The methods enable us to more readily assess wines for possible contamination.

The Viticulturist responded to 415 enquiries, participated in nine Institute Roadshows and five *Research to Practice*™ workshops across three states.

The Analytical Service laboratory underwent a technical assessment by NATA to the new ISO/IEC 17025 standard. Two new test methods; namely an enzymatic acetic acid assay and the HPLC multiresidue assay were accredited and two additional staff members have become NATA signatories.

A new Analytical Service fee schedule has been printed as a result of a review of the service delivery charges. In addition, an extensive market research report was commissioned to establish how we were meeting our client expectations. This report has been extremely positive, with no identifiable areas of concern. The results are consistent with increases in revenue in the past financial year. A large proportion of the Analytical Service activities are now associated with customised non-routine projects for both Australian and overseas companies.

Eleven thousand copies of the Institute's annual publication, *Agrochemicals registered for use in Australian Viticulture 2003/2004* were produced in response to an increase in demand. The reduction in the number of agrochemical related enquiries in comparison to previous years illustrates the effectiveness of the Institute's role in compiling and distributing this publication.

A booklet entitled *The A-Z of information on wine and health* was developed and published. The Commonwealth Department of Health and Aged Care favourably reviewed the booklet and contributed towards its publication and printing costs. As of 30 June 2003, approximately 48,500 booklets have been distributed.

Staff of the John Fornachon Memorial Library responded to 3,608 requests for information during 2002/2003.

Over 5,000 new records were added to the web-accessible database of the Library (available only to Australian winemakers and grapegrowers) during the year, making a total of over 25,000 records available for searching, 24 hours per day, 7 days per week.

Six issues of Technical Review published during the 2002/2003 financial year were consolidated onto a searchable CD ROM and distributed to Australian winemakers and grapegrowers. The new website of The Australian Wine Research Institute was launched to industry. The new website is easier to navigate and faster to download and contains additional useful information.

General highlights

The Institute published 41 papers on Institute activities in refereed and non-refereed publications.

Institute staff gave 174 oral presentations, conducted thirteen workshops and presented 20 posters.

Institute staff presented 48 lectures and coordinated a six week subject to undergraduate students.

Institute staff supervised/co-supervised 31 postgraduate students.

Institute staff recorded and responded to 6,001 requests for information during the 2002/2003 year or, to put the statistics into perspective, 24 people contacted the Institute seeking information on every working day of the year. This figure does not include request for work through the Analytical Service which conducted 54,000 individual analyses during 2002/2003.

Readers are strongly encouraged to read the report in detail rather than relying on the points above for information.

Staff activities

In addition to undertaking research and other projects described in this report, the Institute performs a large number of external activities in support of the Australian wine industry.

Information on seminars, talks and poster papers given to outside organizations, academic lectures delivered, graduate students supervised, and the papers published is tabulated and can be found in Appendices 1–4 of the Annual Report. Activities in addition to those in the Appendices are described below.

Peter Høj is a member of the following:

Prime Minister's Science, Engineering and Innovation Council
Premier's Science and Research Council (South Australia)
Premier's Wine Council (South Australia)
Compliance and Technical Advisory Committee (AWBC)
Technical Committee (Winemakers' Federation of Australia)
Provisor Pty Ltd Board
Cooperative Research Centre for Viticulture II (CRCVII) Board
Wine Committee (Royal Agricultural and Horticultural Society of South Australia)
Waite Campus Management Committee
Committee of Management, Viticultural Publishing, publisher of *Australian Journal of Grape and Wine Research*
Editorial Board of the *Journal International des Sciences de la Vigne et du Vin*
Conference Planning Committee of the Twelfth Australian Wine Industry Technical Conference (24-29 July 2004, Melbourne) (Chair)
Review Committee (into collaboration between Universities and publicly-funded research agencies)

He is also the Institute's representative on The University of Adelaide's School of Agriculture and Wine's Advisory Committee and the Management Committee. Professor Høj is the current holder of the Australian Wine Industry Chair of Oenology at The University of Adelaide.

Sakkie Pretorius is a member of the Editorial Board of the following journals: *American Journal of Enology and Viticulture, Annals of Microbiology, FEMS Yeast Research* and *Yeast*. He is also the Co-Chair of the Program Sub-Committee of the Twelfth Australian Wine Industry Technical Conference, and member of the Conference Planning Committee. He is also an Affiliate Professor of the University of Stellenbosch.

Hans Muhlack is the Public Officer of the Australian Wine Industry Technical Conference Inc. He is also the Company Secretary and Public Officer of Provisor Pty Ltd.

Rae Blair is a member of the Conference Planning Committee for the 12th AWITC and is the Treasurer and Conference Manager of the Australian Wine Industry Technical Conference.

Creina Stockley is a Member, Wine Industry Technical Advisory Committee (as Technical Liaison)
Member, AWBC Legislation Review Committee
Member, AWBC/WFA Wine Industry National Environment Committee
Member, Eco-efficiency Working Group (Sub-committee of SAWBIA's Environment Committee)
Member, Australian delegation to the Office de la Vigne et du Vin
Vice-President, Nutrition and Wine Expert Group of the Office de la Vigne et du Vin
Member, Scientific Advisory Board, Vinsalud Chile 2002 Wine and Health International Congress
Member, Waite Campus Executive Committee
She is also a Board Member of The University of Adelaide's Children's Services.

Elizabeth Waters is Manager of Program 1 of the CRCVII.

Mark Sefton is on the editorial review board of the *International Journal of Vine and Wine Sciences* and is the project leader of project 1.3 of the CRCVII.

Leigh Francis is an Editorial Board member of the *Journal of the Science of Food and Agriculture.*

Markus Herderich is Leader of Project 1.2, the 'Tannin project', of the CRCVII, Affiliate Associate Professor at The University of Adelaide and he is a member of the Advisory Board of the *Journal of Agricultural and Food Chemistry.*

Paul Henschke serves on the Editorial Review Board of the following journals: *Australian Journal of Grape and Wine Research; South African Journal of Enology* and *Viticulture and Mitteilungen Klosterneuburg.* He was a member of the 2nd Australian Conference on Yeast: Products and Discovery, Melbourne (27-29 November 2002) (Chair 2003/2004).

Eveline Bartowsky served on the Faculty of Sciences (Waite Campus) Occupational Health and Safety Committee.

Peter Godden is a member of the 12th AWITC Conference Planning and Program Sub-Committees and is the Workshop Coordinator of a program of ~70 workshops to be held at the Twelfth Australian Wine Industry Technical Conference.

Mark Gishen is leader of project 1.4 of the CRCVII, and is the Institute's representative on the Winemakers' Federation of Australia (WFA) and Winegrape Growers' Council of Australia's (WGCA) recently formed working group known as the Legal Metrology Group.

Don Buick is a voluntary assessor for the National Association of Testing Authorities in the field of chemical analysis of food and wine.

Matthew Holdstock serves on the Interwinery Analysis Group Committee.

Rachel Edwards, Rhonda Packer, Emma-Kate White and Julie McConnell

Visitors to the Institute

Australia

Chris Proud, E&J Gallo (19 July 2002)
35 members of the Clare Valley Vine Improvement Association (19 August 2002)
James McWha, Vice Chancellor, **Edwina Cornish,** Deputy Vice-Chancellor, Research, **Paul Duldig,** Director of Finance, The University of Adelaide (13 September 2002)
Paul Baggio, Australian Winemakers, (24 September 2002)
Richard Gibson, Scorpex Wine Services (24 September 2002)
Dr Michael Perkins and 10 medicinal chemistry students, Flinders University (27 September 2002)
Hutch Rank, Managing Director, **David McQuinn,** Development Manager, **Nick Weckert,** District Manager, **David Richards,** Sales Consultant, DuPont (16 October 2002)
Dr Janet Gardiner, Australian Ambassador to Portugal and **David Haynes,** Executive Officer Administration and Business Relations, Foreign Affairs and Trade (22 October 2002)
Catherine Murphy, Chief of Staff, Office of the Minister for Education Science and Training (Dr Brendan Nelson) (1 November 2002 and 24 April 2003)
Keith Evans, Director, Drug Strategy and Programs Branch, Department of Human Services (5 November 2002
Dr Raymond Mawson, Dr Kamaljit Singh Vikhu, Dr Cornelis Versteeg, Food Science Australia (12 December 2002)
Barbara Hardy, AO and **Bill Hardy** (16 December 2002)
Mario Marson, Grapegrower Heathcote/Jasper Hill Vineyard (30 December 2002)
Norm Cook, Dupont (13 January 2003)
Dr Lawrie Besley, National Analytical Reference Laboratory (7 February 2003)
Steve Morton, Executive Officer, Premier's Science Council (10 February 2003)
George Krackljack, ARRM (20 February 2003)
George Bowyer and **Greg Byrne,** BioStart (26 February 2003)
Professor Ken Watson, School of Biological, Biomedical and Molecular Sciences University of New England (12 March 2003)
Ian Sutton, Winemakers' Federation of Australia (20 March 2003)
Jodie Goode, Michael Shillabeer, SA Department of Business Management and Trade (2 April 2003)
George Willcox, Vaslin Bucher (24 April 2003)
Jane Evans, Technology Transfer Manager, CRC for Bioproducts (28 April 2003)
Bryn Haugaard, Austwine Exports Pty Ltd (29 April 2003)
Hon John Hill MP, Minister for the Environment, **Mike Young,** CSIRO, **Peter Hoey,** Director, Murray Darling Division, Dept of Water Resources, **Simon Divecha,** CEO Conservation Council of SA, **Senator Penny Wong** and staff member **John Olenich, Martin Ferguson** MP, Shadow Minister for Regional Development, **Murray Radcliffe and Denise Spinks** (Staff of Martin Ferguson), **Alex Gordon,** Environment Adviser, Office of Simon Crean, **Dick Adams MP** – Tasmania, **Kelvin Thompson MP,** Shadow Environment Minister, **David Cox MP,** SA MP and Shadow Assistant Treasurer, **Michael Hatton MP** – NSW (2 May 2003)
Roger Hoare, Grape and Wine Research and Development Corporation (8 May 2003)
John Power, Assistant Manager, Wine Policy, Department of Agriculture, Fisheries and Forestry Australia (16 May 2003)
David Edmonds, National President; **Ken Pidgeon,** SA Brand President, Royal Australian Chemical Institute. (2 June 2003)
Willy Billiard, freelance journalist, Qld. (6 June 2003)

International

Denmark

Ole Vestergaard, Aahus University, Denmark (27 February 2003)
Laura West, De Danske Spritfabrikker, Denmark (29 April 2003)

Chile

Danilo Sturiza Jordan, Director Zonal; and **Veronica Godoy,** Investment Specialist, O'Higgins Regional Branch; TodoChile, **Alejandro Soto,** Regional Director CORFO Maule Region, Chile (8 July 2003)

France

Dr Veronique Cheynier, Unité Mixte de Recherche Sciences pour l'Oenologie: Equipe Polyphénols, INRA (9 October 2002)
Dr Vassela Atanasova, Unité Mixte de Recherche Sciences pour l'Oenologie: Equipe Polyphénols, INRA (10 March 2003)
Patricia Villavicencio, ADEPTA, France (24 April 2003)
Francois Davaux, ITV, France (24 April 2003)
Dr Jean-Claude Ruf, Office International de la Vigne et du Vin, France (12-13 December 2003)

Germany

Dr Oliver Schmidt, Lehr- und Versuchsnstalt fur Wein-und Obstbau Weinsberg Weinsberg, Germany (25 July 2002)

Greece

Dr Vassilios Marinos, Chemist-Oenologist, Ampeooeniki, Greece (6 February 2003)

Hungary

Lazlo Romsics, Hungary (10 June 2003)

Italy

Sam Benelli (Sales Manager, Oenological Products) and **Dr Giuliano Boni** (Sales Support Manager, Oenological Products), Esseco SpA, Italy (24 September 2002)

Japan

Professor Takesako and five students from Meiji University, Japan (6 September 2002)
Keita Hori and **Ken Inose,** Okutone Wine KK; **Yamagani Naoshi,** Yumego Budoshu Kenkyusho; **Nasazuni Dobashi,** Chuo Budoshu; **Noriko Kishidaira,** Takeda Winery; **Hisayoshi Tsuiki,** Katunuma Jozo; **Kozo Tuchiya,** Kizan Yoshu; **Sadahiro** and **Kazuhiro Yamada,** Hojo Wine; **Kenzo Akazawa,** Yamato Budoshu; **Mashami Hayakawa,** Hayakawa Co. Japan, (18 February 2003)
Yoshiyuki Tateishi, Kirin Brewery Co Ltd, Japan (8 May 2003)

New Zealand

Paul Kilmartin, Lecturer in Analytical Chemistry & Food Science, Auckland University, New Zealand (25 November 2002)
Gerard Besamusca, AgConsult, New Zealand (26 February 2003)

Portugal

Associate Professor Maria-Theresa Ribeiro de Lima, Agricultural Sciences Department of Azorean University, Portugal (24 March 2003)

Thailand

A group of 30 winemakers, organised by **Dr Charoen Charoenchai,** Thailand (17 March 2003)

United Kingdom

Sam Harrop, Marks and Spencer, London, UK (20 August 2002)
Peter Scott, Director Programme Development, CAB International, Oxon, UK (19 September 2002)

USA

Dr Nancy Irelan, E&J Gallo (August 2002)
Dr Jeffrey McCord, StaVin Inc, USA (11 October 2002)
Leslie Norris, FlavorSense, USA (11 October 2002)
Patrick Gleeson, American Vineyard Foundation, USA (7 November 2002)
Dr Mary Wagner, Chief Technology Officer; **Dr Terry Lee; Tom Smith,** Vice President – Wine Growing; **Greg Coleman,** Director – Grower Relations; **Chris Proud,** Director – International Winemaking; E&J Gallo, USA (26 March 2003)

Research Teams' report

Investigations into the relationship between Dekkera/Brettanomyces yeast and red wine in Australia

Staff: Peter Godden, Professor Peter Høj, Professor Sakkie Pretorius, Jane McCarthy, Jennifer Bellon, Dr Paul Henschke, Dr Peter Costello, Dr Miguel de Barros Lopes, Dr Leigh Francis, Kate Lattey, Dr Mark Sefton, Dimitra Capone, Dr Alan Pollnitz, Mark Gishen, Adrian Coulter, Geoff Cowey, Ella Robinson, Greg Ruediger

Preliminary investigations have been conducted under a number of Institute projects during the current reporting period, as a prelude to a dedicated project that will be funded from the 2003/2004 financial year onwards. Approximately 60 isolates of *Dekkera/Brettanomyces* yeast have been obtained from various Australian wines by the Industry Services team. These isolates have been confirmed as *Dekkera/Brettanomyces* by Dr Miguel de Barros Lopes and Jenny Bellon of the Institute's Molecular Biology team, using Polymerase Chain Reaction (PCR) analysis. Initial amplified fragment length polymorphism (AFLP) analysis indicates that although approximately 50% of the isolates are genetically very similar, a considerable degree of genetic diversity exists between the isolates. It is intended that the most genetically diverse isolates will subsequently be used in small-scale fermentation experiments. This aspect of the project will seek to investigate any differences between strains in their propensity to form 4-ethylphenol, 4-ethylguaiacol and another reported important spoilage compound, isovaleric (3-methyl butyric) acid, when varying concentrations of precursor compounds and nutrients such as residual sugars and nitrogen, are present. The effect of pH and SO_2 concentration on the ability of strains to form the reported spoilage compounds and their correlation with the intensity of the 'Brett' character will also be investigated.

The development of a GC-MS method for the quantification of isovaleric acid (3-methyl butyric acid) and 2-methyl butyric acid, is reported under *Analytical method development and evaluation.* Whilst isovaleric acid is reported in the literature to be an important spoilage compound formed by *Dekkera/Brettanomyces* yeast, initial analytical results obtained using this analytical method indicate no correlation between the concentrations of 4-ethylphenol and isovaleric and 2-methyl butyric acids albeit in a small number of wines (Table 1). However, it is possible that synergistic sensory effects exist between these compounds, which *may* result, for instance, in the presence of isovaleric acid enhancing the apparent intensity of other *Dekkera/Brettanomyces*-derived taints in wine. It is considered of the utmost importance that such sensory relationships are elucidated and, therefore, sensory investigations will be a major part of the ongoing *Dekkera/Brettanomyces* project.

Table 1. 4-Ethylphenol and 3-methyl butyric acid concentrations (µg/L) of Cabernet Sauvignon wines produced by two Australian wineries 1996 – 2001

	Winery A		Winery B	
Vintage	4-ethylphenol µg/L	3-methyl butyric acid (isovaleric acid) µg/L	4-ethylphenol µg/L	3-methyl butyric acid (isovaleric acid) µg/L
1996	NA	NA	1500	1654
1997	1420	973	2450	1095
1998	1730	1231	2130	1274
1999	393	1383	2180	1469
2000	603	1521	563	1489
2001	32	1484	333	1311

The data in Table 1 demonstrate that it is possible for wineries to achieve substantial reductions in 4-ethylphenol concentrations by implementing winemaking strategies to control the proliferation of *Dekkera/Brettanomyces* yeast. It is pleasing to Industry Services staff that, for instance, winery A in Table 1 was one of the first to contact the Institute regarding *Dekkera/Brettanomyces* control, late in 1998. During 1999 and subsequently, winery A and Institute staff have had many discussions on the same issue. Similarly, winery B in Table 1 first contacted Industry Services regarding the same issue early in 2000. A seminar relating to *Dekkera/Brettanomyces* control has been presented to approximately 1500 winemakers in a number of forums, particularly Institute Roadshows, over a period of approximately four years. Additionally, the *Trouble free winemaking* workshop conducted by the Institute, has a strong emphasis on the control of microbiological instabilities including *Dekkera/Brettanomyces,* and has been presented on a total of 15 occasions since it was first developed in 1999.

The two wineries for which data are presented in Table 1 are noteworthy for the magnitude of the reduction in 4-ethylphenol concentrations that they have been able to achieve. However, an ongoing survey being conducted by Industry Services provides some evidence that a reduction in median 4-ethylphenol concentration occurred in Cabernet Sauvignon and Cabernet Sauvignon/Merlot wines produced in five regions during the 2001 vintage, compared with the previous five vintages. Wines from the regions Barossa Valley, Coonawarra, Hunter Valley, Margaret River and Yarra Valley have been surveyed in a representative manner. One hundred and seventy-seven wines from the vintages 1996 to 2000, and 51 wines from the 2001 vintage have been analysed. There were no significant differences in the median or mean (data not shown) concentrations of 4-ethylphenol for the vintages 1996 to 2000. However, the mean concentration of 4-ethylphenol in wines from the 2001 vintage, compared to the mean of the pre-2001 wines, fell by 46% (data not shown). It is also of interest that the distribution of 4-ethylphenol concentrations appears to have changed in 2001 compared to the previous four years, with the lower approximately 62 percentile of concentrations in 2001 falling in the range occupied by the lower 25 percentile of concentrations of the previous four years. Whilst these results are encouraging, care should be taken when interpreting these

data, as it is possible that climatic or other factors during the growing season and winemaking might account for the apparent fall in the concentration of 4-ethylphenol in Cabernet Sauvignon and Cabernet Sauvignon/Merlot wines from these five regions in 2001. The inclusion of data from the much cooler 2002 vintage (in most regions) will help address this issue.

*See further report on the Institute's work with *Brettanomyces* under Dekkera/ Brettanomyces - *isolation from wine and a preliminary physiological character isolation* which appears later in this annual report.

Wine grape tannin and colour specification

Staff: Dr Markus Herderich, Dr Leigh Francis, Dr Liz Waters, Dr Zhong Kui Peng (until August 2002), Dr Paul Smith (from May 2003), Dr Stéphane Vidal (until November 2002), Dr Patrik Jones (from April 2003), Dr George Skouroumounis, Yoji Hayasaka, Gayle Baldock, Maria de Sa, Heather Donnell, Holger Gockowiak (appointed as part-time Technical Officer in July 2002), Mariola Kwiatkowski, Kate Lattey, Kevin Pardon, Mango Parker (nee Ranzijn).

Collaborators: School of Agriculture and Wine, The University of Adelaide: Dr Patrick Iland (continues as consultant after resignation from The University of Adelaide in July 2002), Dr Graham Jones, Stephanie Lambert (PhD completed October 2002), David Lee, Renata Ristic, Dr Ewald Swinny (appointed as postdoctoral research fellow in August 2002). Institut National de la Recherche Agronomique, Montpellier France: Dr Veronique Cheynier

The aim of the 'Tannin project' is to understand the basis of red wine colour at a molecular level; to identify compounds that are relevant for specific mouth-feel properties; and to develop a detailed insight into all transformations of grape compounds which impact on wine composition, quality and style. During the last year, our research into wine grape tannin and colour specification has continued with a focus on detection and identification of polyphenols important to red wine colour and mouth-feel, and of conditions favouring their formation during winemaking and ageing.

Dr Paul Smith commenced employment in May 2003 as Research Chemist. Paul will focus on structure elucidation of wine tannins and pigmented polymers, and aim to determine the molecular basis for mouth-feel and colour properties of polyphenols present in red wine. Paul will cooperate closely with Dr Patrik Jones, who commenced in April 2003 as Research Chemist and as the replacement for Dr Stéphane Vidal with funding available through the CRCV. Patrik's research will concentrate on flavour/ mouth-feel interactions, with a strong element focussing on tannins and other phenolic compounds in wine. With the appointment of Holger Gockowiak as part-time Technical Officer we are consolidating our efforts in the preparative isolation of tannins and anthocyanins and Holger will help us with the purification of crucial reference compounds and essential starting materials for sensory studies. The remodelled team made very good progress as evident from its many publications which appeared during the year (Appendix 4), and team members were invited to present the outcomes of the 'Tannin project' to the scientific community at international conferences and efficiently discussed the emerging knowledge with Industry during Roadshow seminars (see Appendix 1).

Analytical method development

A new gradient HPLC system has been installed and commissioned, and we have now three fully operational systems available for analytical method development and routine analysis of anthocyanins, tannins and 'pigmented polymers' (note that throughout this report 'tannin' refers exclusively to 'proanthocyanidin' or 'condensed tannin'). Together with our improved, rapid and robust methods for HPLC analysis, this investment in infrastructure enabled us to efficiently handle more than a thousand research samples from viticultural and winemaking trials, and research projects of the Tannin, NIR and Wine Microbiology teams.

To improve the efficiency of our analytical operations further, we continued to evaluate the potential of near infrared (NIR) spectroscopy for the rapid measurement of phenolic compounds in red wine fermentations in collaboration with the NIR team. HPLC reference data from a total of 495 samples from 32 commercial-scale red wine fermentations over two vintages using two grape varieties (Cabernet Sauvignon and Shiraz), and also including as additional variables two types of fermenters, two different yeasts, and three fermentation temperatures were used to develop NIR calibrations. With this collaborative study, we demonstrated the potential of NIR spectroscopy for rapid prediction of phenolic compounds in red fermentations without performing exhaustive pre-treatments or sample preparation. However, more experiments are required to determine the chemical specificity of the calibrations. For the isolation and purification of anthocyanins and 'pigmented polymers',we

Figure 1. Colour properties of the grape anthocyanin malvidin-3-glucoside and anthocyanin-derived pigments. (a) under acidic conditions at pH 1.5, (b) in model wine at pH 3.6, (c) same as (b) but in presence of SO_2.

continued to explore the potential of multi-layer coil countercurrent chromatography (MLCCC). For the first time, we applied solvent step-gradients with MLCCC separations and optimized the isolation of anthocyanins and coumaroylated anthocyanins from several commercially available grape marc extracts. Equally important was the observation that very polar polymeric pigments, which are now the focus of structure elucidation efforts, could be enriched in the aqueous stationary phase upon MLCCC separation. In addition, 'pigmented polymers' have been isolated by MLCCC for sensory assessment. These pigmented tannins were less astringent than the smallest native tannins from apple, grape seed and grape skin that have been tasted by our panels. While this very interesting result could suggest that anthocyanins might carry their lack of mouth-feel properties with them into polymeric compounds, future research in this area is required to confirm the supposedly reduced astringency of 'pigmented polymers' and to establish whether concentration effects, molecular mass and structural properties could contribute to these modulated mouth-feel properties.

Characterisation of red wine pigments, 'pigmented polymers' and tannins

As reported previously, tandem mass spectrometric experiments had allowed us to detect numerous potential red pigments in wine samples (Institute publication #678). For the first time, these malvidin-derived pigments have now been synthesised and we could demonstrate that the formation of the pigments from vinylphenols involved a complex combination of addition, ring closure and oxidation reactions. With the help of the synthesised reference compounds we confirmed the structural assignment by extensive NMR experiments. We also demonstrated (Figure 1) that these wine pigments were almost inert to SO_2-mediated bleaching while grape anthocyanins such as malvidin-3-glucoside could be instantly decolorized, and that these wine pigments showed superior colour properties and enhanced extinction coefficients in model wine at pH 3.6 when compared to anthocyanins. In summary, this research demonstrated how grape anthocyanins can be transformed into stable and unbleachable red pigments, most of which are likely to be formed during winemaking and ageing (Institute publication #725).

Similar to the above-mentioned reaction of malvidin-3-glucoside with vinylphenols, the formation of the anthocyanin-derived wine pigment vitisin-A was studied by 'Tannin project' members Robert Asenstorfer and Graham Jones (The University of Adelaide) with fermentation trials. This research demonstrated that an oxidation step was required, similar to the reaction of malvidin-3-glucoside in presence of vinylphenols studied by the Institute's team (Asenstorfer, R.E.; Iland, R.G.; Markides, A.J.; Jones, G.P. [2003] Formation of vitisin A during red wine fermentation and maturation. Aust. J. Grape Wine Res. 9, 40 – 46). The same authors published a manuscript summarizing their research on structures and colour properties of malvidin-3-glucoside at various pH values and provided evidence that an uncharged quinoidal isomer could represent the major coloured isoform of malvidin-3-glucoside at wine pH (Asenstorfer, R.E.; Iland, P.G.; Tate, M.E.; Jones, G.P. [2003] Charge equilibria and pKa of malvidin-3-glucoside by electrophoresis. Analyt. Biochem. 318, 291 - 299).

The development of methods to measure and to characterise tannins and 'pigmented polymers' remained an area of high priority and productive research for the Tannin team. Methods to quantify the levels of tannins and 'pigmented polymers' in seed extracts and in wine have been developed, and the identity of tannins has been demonstrated by mass spectrometric analysis. The outcomes of our study on the structural characterisation of grape seed tannins by electrospray mass spectrometry have been published (Institute publications #660, 686, 713). In collaboration with Dr James Kennedy (Oregon State University, USA) 'pigmented polymer' samples prepared from Pinot Noir wine were characterised by ESI-MS, ESI-MS/MS and HPLC-MS experiments at the Institute. Analysis of the MS data revealed that the 'pigmented polymers' from Pinot Noir were the products of direct condensation of anthocyanins with tannins. Interestingly, we could demonstrate the presence of rather large condensation products with up to eight catechin subunits (octameric tannins) amongst the 'pigmented polymers'. Until now, the existence of such direct condensation products obtained from anthocyanin and larger tannins has been postulated, but never been published to our knowledge.

Understanding the astringency and mouth-feel properties of condensed tannins and related polymers

In addition to the results from studies on sensory properties of tannins and anthocyanins as summarized in Institute publications #688, 697 and 717, we characterised catechin oligomers obtained in the presence of acetaldehyde and conducted, in collaboration with Professor Ann Noble, time-intensity studies for quantifying astringency, bitterness and other mouth-feel sensations. It could be demonstrated that tannin-like catechin oligomers were formed from catechin monomers in presence of acetaldehyde by linkage through ethyl bridges. When tasted, the synthetic catechin oligomers of a mean degree of polymerisation of 5 (mDP 5) had astringency mid-way between that of native catechin oligomers of mDP 3 and mDP 9 linked through direct inter-flavan bonds. This result suggested that the number of subunits in tannins could have a bigger influence on their astringency properties than the type of linkage between units.

The sensory properties of anthocyanins were revisited and purified anthocyanin fractions have been tasted in model wine (13% ethanol in saturated aqueous potassium hydrogen tartrate solution at pH 3.6) as part of a descriptive sensory analysis undertaken at the Institute and in 5% ethanol as part of a paired comparison study in Montpellier. In both studies these purified anthocyanin fractions were not perceived as bitter or astringent, nor did they influence the fullness or viscosity of the solutions they were tasted in. It is likely that the low level of astringency and fullness perceived initially with less pure fractions was due to minor impurities of other phenols rather than the anthocyanins themselves.

In summary, the investigations that have been carried out in collaboration with Dr Cheynier's group at the Institut National de la Recherche Agronomique in Montpellier are very valuable additions to our previously scarce knowledge regarding what components can contribute particular mouth-feel characteristics when a wine is tasted. Although the experiments were carried out in model systems, and further work is required to confirm these effects in actual red wines, the results point to some important advances.

As might be expected, tannin composition and concentration influenced astringency most strongly, with the findings demonstrating that larger tannins were more astringent at a given mass (rather than molar) concentration than smaller tannins and that seed tannins were perceived as more coarse and astringent than skin tannins of comparable size. The grape pigments, anthocyanin glucosides and their corresponding coumarates, were not perceived as bitter or astringent. In addition, they did not influence the fullness or viscosity of the solutions they were tasted in. It remains to be confirmed whether anthocyanins carry their lack of mouth-feel properties with them when they are incorporated into oligomeric and polymeric structures, and whether condensation of tannins with anthocyanins could reduce tannin-related astringency. With regard to the effect of polysaccharides, the two types studied conferred the sensation of fullness in the absence of tannins, but not in the model system with tannins present.

Winemaking practices that affect tannin concentrations, and colour and sensory properties of wine

A sensory study involving difference testing and descriptive analysis of wines from the 2001 vintage commercial-scale winemaking experiments has been completed. Notably, for all of the 18ºC ferments with Shiraz or Cabernet Sauvignon, the wines made in the Potter fermenter were more astringent (drying, coarse and adhesive) than those fermented in the rotary tanks. This effect was not evident for the higher temperature (25ºC) ferments, where the two fermenter types gave wines with similar mouth-feel properties. One likely explanation for this 'Potter effect' could be linked to the oversized centrifugal pumps used for pump-over in 2001 that were likely to enhance extraction by mechanical stress on the solids. There is, however, no conclusive explanation available at this stage for the 'Potter effect', as wines made in Potter fermenters at 25ºC also featured enhanced extraction of phenolics when compared to wines made in rotary fermenters at 25ºC, while lacking the differences in astringency. These observations need to be confirmed but nevertheless demonstrate that precise quantitative measurements of tannins are as essential as methods to characterise tannin structures. To identify the basis for the perceived mouth-feel differences in these wines we will now explore the correlation between tannin concentration, tannin structure and astringency levels in greater detail.

In collaboration with Institute microbiologists (Dr Paul Henschke, Dr Eveline Bartowsky, Jeff Eglinton), the influence of yeast strain choice on tannin and colour properties was

pursued. The wines from the joint 2002 commercial-scale winemaking trial comparing *S. cerevisiae* with *S. bayanus* have been analysed by HPLC and NIRS twice daily during fermentation until pressing, during MLF and after bottling. Statistical analysis of the HPLC data for malvidin-3-glucoside as the dominant grape-derived anthocyanin and 'pigmented polymers' formed during fermentation has demonstrated that by using rotary fermenters at 20°C all wines made with *S. bayanus* had less malvidin-3-glucoside but more 'pigmented polymers', and also more tannins compared to wines made with *S. cerevisiae*. Whether fermenting with *S. bayanus* enhanced tannin extraction and/or resulted in enhanced formation of tannin-like polymers remains to be clarified. As these red wines have been made from carefully randomized grapes under controlled and replicated conditions, we are now planning sensory studies with focus on the 'yeast-effect' on wine composition, wine tannin concentration and astringency properties.

In addition to these commercial-scale fermentations with *S. bayanus,* we studied the chemical reactivity of anthocyanins with grape-derived oenotannins in model reactions in the presence of different SO_2 and acetaldehyde concentrations. Addition of both SO_2 and acetaldehyde to anthocyanin/tannin mixtures resulted in reduced formation of 'pigmented polymers' when compared to model reactions with acetaldehyde alone, but still enhanced 'pigmented polymer' formation when compared to the model reactions between anthocyanins and tannins in presence of SO_2 alone. In summary, acetaldehyde was identified as one likely yeast metabolite that could contribute to enhanced formation of 'pigmented polymers' from anthocyanins and tannins, and we are now aiming to measure acetaldehyde on a regular basis during red wine fermentations with the help of a GC-MS method that has recently been validated.

Our main objective with the on-going 2003 Tannin trial is to study the impact of tannin additions (200 ppm commercially available 'colourless', proanthocyanidin-based grape seed tannins) on red wine phenolic composition, tannin concentration, colour and mouth-feel properties. With the current trial we are aiming to demonstrate the effects of adding the oenotannins pre- or post-fermentation to Potter fermenters. We also expect to ascertain whether our existing analytical methods are specific enough to identify the molecular basis for the perceived colour and mouth-feel changes. The grapes for the 2003 large scale winemaking trials of the 'Tannin project' and the Institute's microbiology team have been provided by Banrock Station and the excellent support from Ben Vagnarelli and his team is gratefully acknowledged. After crushing of the grapes in the Hickinbotham Roseworthy Wine Science Laboratory, the Tannin team members collaborated closely with the Institute's Wine Microbiology and NIR teams to foster research synergies and to minimize trial-related costs. This approach has already been proven successful during the 2002 vintage, and it enabled us to analyse all twelve fermenting tanks during alcoholic fermentation on a daily basis. Comprehensive data were collected for phenolic composition by HPLC and NIR spectroscopy. At present, all wines are undergoing malolactic fermentation and we will continue to analyze the samples at bottling and after approximately twelve months with the sensory studies.

NIRS project: rapid instrumental techniques

Staff: Mark Gishen, Dr Bob Dambergs, Dr Leigh Francis, Dr Wies Cynkar, Professor Peter Høj, Dr Elizabeth Waters, David Boehm, Les Janik, Dr Daniel Cozzolino

Collaborators: Chris Bevin, Audrey Lim (Hardy Wine Company), Russell Johnstone, Inca Lee (Orlano Wyndham), Dr Eric Wilkes (Beringer Blass), Dr Bruce Kambouris (McGuigan Simeon)

Research into rapid instrumental methods has continued to concentrate on pursuing the promising technique of near infrared (NIR) spectroscopy. This analytical approach has been shown from earlier work to be capable of providing very fast, low cost analyses of a range of parameters important to commercial wine production. Spectroscopic techniques offer the potential to simplify and reduce analytical times for a range of grape and wine analytes. It is this aspect, together with the ability to simultaneously measure several analytes, which was the impetus for developing NIR methods.

In response to the wine industry's need for rapid analytical methods for the determination of objective indicators of grape quality, a decision was made to concentrate efforts on development of an NIR method for determining colour, total soluble solids (TSS) and pH in red grapes, that would be suitable for practical industry use. Several reports and Roadshow presentations by the project team have introduced the potential use of NIR for red grape quality assessment and a detailed article has been published in the 2003 *Australian & New Zealand Grapegrower & Winemaker* Technical Issue (Institute publication #730). Several large wine companies have now put this technology into practice.

It has been proposed that transfer of the technology to the industry might best be achieved through direct commercialisation activities, and this is being carried out under the responsibility of the Cooperative Research Centre for Viticulture's dedicated commercialisation company, CRCV Technologies Ltd. CRCV Technologies Ltd., with the assistance of a commercialisation consultant, have engaged in negotiations with several instrument manufacturers in their management of trial commercial release of instruments provided with calibrations developed by the Institute.

The consolidation of calibrations developed for the analysis of grape berry colour, total soluble solids and pH by NIR scanning has continued, involving study of as wide a range of samples as possible. The continued cooperation of industry partners has meant that approximately 3000 berry samples from the 1999, 2000, 2001, 2002 and 2003 seasons from a wide range of growing regions and representing several red varieties (but predominantly Shiraz and Cabernet Sauvignon) have been analysed by the conventional laboratory methods and scanned with a research grade NIR instrument located at the Institute. The berry samples were scanned with no preparation other than homogenisation using a high-speed laboratory homogeniser.

It has been found previously that, while the calibrations appeared to hold across seasons and a calibration could be developed that will give acceptable results for samples from multiple regions or from several varieties, best accuracy seemed to be obtained from restricted sample sets (e.g. same vintage, region or variety). It has been shown that a regression method known as LOCAL could dramatically reduce the prediction error for grape colour from large, diverse data sets, but a simple method may be to tailor standard partial least squares (PLS) calibrations for samples of similar colour values. Table 2 compares calibration statistics for the full 1999 to 2001 dataset with subsets selected by colour range, irrespective of vintage, grape variety or growing region. A mid-range colour set (colour between 0.5 and 1.5 mg/g) had a lower standard error of cross validation (SECV) than the full data set and the equation developed with this mid-range set had an increased standard error of prediction (SEP) when predicting the extreme range samples. Note also that an equation developed with 20% of the mid-range set could predict the remaining 80% with better accuracy than the full data set, thus with a large dataset, analytical workload can be reduced by using an NIR method. Sample storage and processing are important issues that require consideration when performing analysis on grape samples. The effect of freezing and homogenisation of red grapes on the determination of total anthocyanins, TSS and pH was determined recently. Three different commercial homogenisers were used for sample preparation. The effects of freezing, homogeniser type and storage time on the accuracy and recovery of the laboratory determination and NIR prediction of colour, TSS and pH are being evaluated. Preliminary results have indicated that neither freezing of samples prior to homogenisation, nor the type of homogeniser had any significant effect on the accuracy of laboratory determinations of colour and TSS. However, as one might expect, there was an effect of freezing on pH. This study is continuing with further samples remaining in frozen storage for later analysis to investigate any long-term stability effects as empirical evidence indicates long-term freezing at -20°C can lead to colour loss. This study will provide the basis for updating the protocols for the analysis of red grapes by both reference and NIR methods.

The Institute has been involved in the testing and assisting in the development of an automated grape sample preparation system 'Bioprep5 Robotics', which has been released commercially by ARRM, a local Adelaide company. The unit is designed primarily to process grape samples for colour analysis, whether by NIR or by the reference laboratory method, but it can be put to other uses. The benchtop-sized unit is fully automated, carrying out grape sample homogenisation, sub-sampling, weighing, addition of an extraction solvent, mixing during extraction, and finally centrifugation, and is multitasking. Following extensive evaluation at the Institute of the commercial system, the unit has been found to perform well in all tests, including those with sets of grape berry samples with challenging physical properties. In recent testing, 156 grape samples were processed and analysed. The data from the automated system compared well to the results obtained from the manual method in terms of accuracy and precision. No serious practical deficiencies were observed in the testing, with aspects such as ease of access to the parts of the unit, ease of cleaning and control of the processing being satisfactory. The unit could be configured to act as an automated homogeniser, with no further processing, to allow NIR scanning of the samples only (i.e. with no reference analyses), in which case the system could process approximately 112 samples/8 hour day. For full processing (including extraction and clarification) with one operator carrying out other analytical tasks on the samples, 48 samples would be the likely maximum that could be processed in an 8-hour period.

The project has continued to investigate the effect of sample presentation in NIR analysis, comparing homogenised grapes with whole red grapes. Initial work by the project team on the prediction of quality parameters in red grapes using NIR began with the scanning of homogenized grape samples using a research grade laboratory NIR spectrometer, the *FOSS NIRSystems 6500*. However, the recent availability of faster scanning Vis/NIR spectrometers with alternative optical configurations has provided the possibility of presenting the grape samples to the instrument without the need for homogenisation. Simplifying the sample presentation for NIR prediction of colour, TSS and pH could dramatically increase sample throughput. Furthermore, this mode of presentation might even offer the potential of scanning whole, single berries. Preliminary investigations for whole grape berry presentation were promising, indicating that NIR may have potential for use at the weighbridge or for in-field analysis. These studies have been performed using several compact, fast, diode-array spectrometers including the *Zeiss Corona*. Since the 2001 vintage, the NIR team has been collaborating with the Tannin Project team in their investigations of process scale fermentations. The main objective was to examine the potential of NIR spectroscopy to predict the concentration of phenolic compounds and monitor the extraction and evolution of these compounds during red wine fermentation. If successful, NIR spectroscopy techniques may offer potential as a rapid, low cost and non-invasive tool for monitoring the fermentation process. The preliminary results from the 2001, 2002 and 2003 winemaking trials conducted at the Hickinbotham Roseworthy Wine Science Laboratory showed that NIR spectroscopy could predict the concentration of malvidin-3-glucoside (the major anthocyanin in grapes), 'pigmented polymers' and tannins in Cabernet Sauvignon and Shiraz wines during fermentation. However, the specificity of the calibrations developed must be confirmed, as there are many simultaneous changes occurring during fermentation. Results from a calibration for malvidin-3-glucoside in red wines from 2001, 2002 and 2003 fermentation trials are shown in Figure 2.

The authentication or identification of food is emerging as one of the most important areas in order to comply with food industry regulations and standards and to address consumers' concerns. Since the early 1980s, the development of multivariate statistical techniques, personal computers, and new instrumentation have resulted in the development of IR methods that offer the possibility to analyse more samples than ever before and acquire large amounts of data. Spectroscopic techniques such as NIR or Fourier transform infrared (FT-IR) have proved to be a suitable tool for authentication with demonstrated applications including discrimination between coffee varieties, honey adulteration, and beef contamination and adulteration. The project team has commenced work using NIR spectroscopy to discriminate between wine varieties and blends. Preliminary investigations have been undertaken into the discrimination of commercial samples of two white wine

Table 2. Calibration statistics for NIRS measurement of colour (mg/g) in samples from multiple vintages, regions and varieties separated by colour range; midrange: 0.5–1.5 mg/g; extreme-range: >1.5, <0.5 mg/g

Calibration set	N	Validation set	SECV	SEP	R^2
All samples, 1999 - 2001	2287	-	0.13	-	0.92
mid-range colour	1618	-	0.09	-	0.89
mid-range colour	1618	extreme-range colour	-	0.16	0.92
20% subset of mid range	324		0.09	-	0.91
20% subset of mid-range	324	80% subset of mid-range	-	0.10	0.87

Notes: N is the number of samples, SECV is the standard error of cross validation, SEP is the standard error of prediction, and R^2 is the coefficient of determination.

varieties (Chardonnay and Riesling), sourced from a PhD project on identifying white wine flavour compounds, conducted by Heather Smyth. Principal component analysis (PCA) of NIR spectra shows discrimination between the two varieties, as shown in Figure 3. Further work is continuing in order to understand the influence of the wine matrix on the classification specificity. It is proposed that red wines will also be investigated using this technique.

In collaboration with the Molecular Biology team, the potential of NIR and MIR spectroscopy to discriminate and identify yeast strains has been examined. Further work has also been planned in collaboration with the Wine Microbiology team in order to assess the suitability of IR spectroscopic techniques to monitor ethanol, sugars and other compounds during laboratory fermentations.

An evaluation of the feasibility of NIR spectroscopy for detection of *Botrytis* and powdery mildew is continuing in collaboration with the Fungal Pathology Laboratory of The University of Adelaide and CSIRO Plant Industry in Canberra. The Fungal Pathology Laboratory has developed a DNA assay for powdery mildew and CSIRO has developed an ELISA assay – these can be used to verify results obtained from NIR scans of infected samples.

Discrimination of two stages of powdery mildew leaf infection (early lesions and sporulating lesions, assessed visually) from control and erinose infected leaves using principal component analysis (PCA) was demonstrated using a small sample set of homogenised leaves. Distinct spectral changes could also be observed in fruit classified into a number of different levels of infection (Figure 4). These spectral changes were used to detect and correctly classify powdery mildew infection down to at least a 10% infection level. Work will now focus on differentiating fungus-induced grape compositional changes from fungal constituents.

Similar work is also in progress with *Botrytis*-infected fruit. The *Botrytis* work has the advantage that it can be done on fruit infected in the laboratory, allowing control of infection at various levels and removing the problem of confusion with secondary infection by other organisms. This work is being done in collaboration with the Fungal Pathology Laboratory of The University of Adelaide.

The implication of this work is that it may be possible to discriminate fungal infected fruit at the weighbridge to provide a 'go/no-go' test to highlight suspect fruit for further detailed analysis to determine suitability for winemaking. Grape assessment for fungal infection at the weighbridge would normally be done by visual inspection, but this can be difficult with mechanically harvested fruit, particularly red grapes.
The project team has continued with

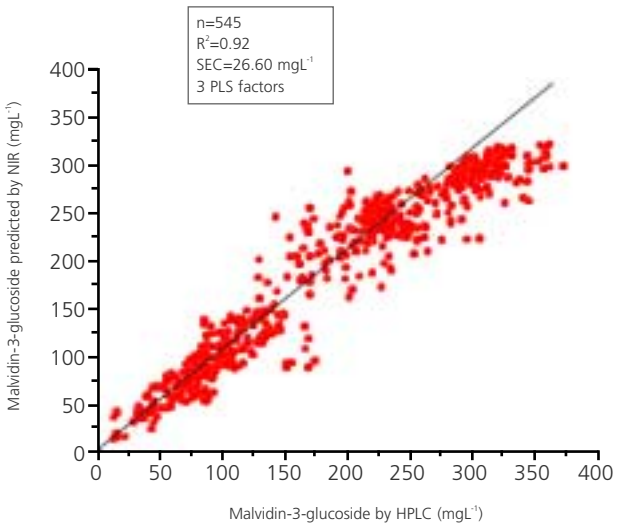


Figure 2. The relationship between NIR-predicted and reference analyisis (HPLC) values for malvidin-3-glucoside in red wine ferments (n: number of samples; SEC: standard error of calibration; PLS: partial least squares)

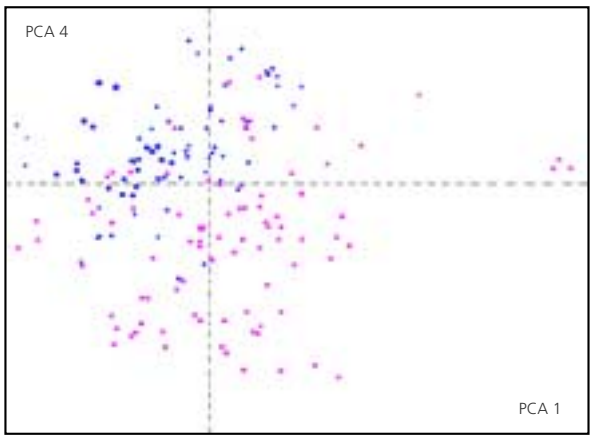


Figure 3. Principal component scores for PC1 and PC4 of NIR spectra, showing discrimination between Riesling (blue) and Chardonnay (purple) wines

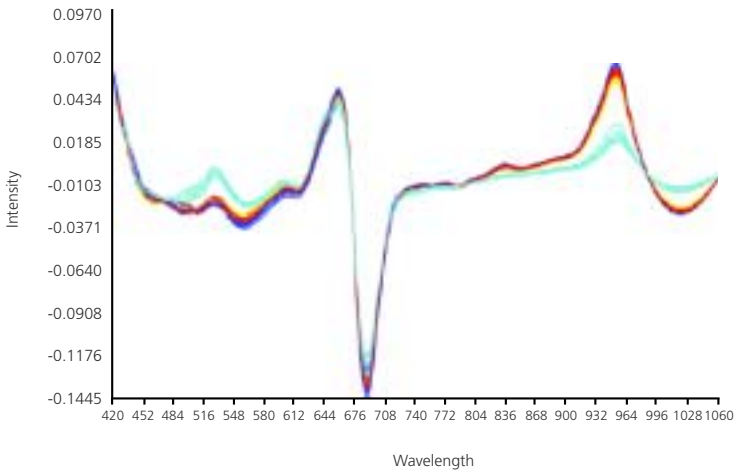


Figure 4. First derivative spectra of uninfected (dark blue), 1-10% (red), 11-60% (yellow), and 61-100% (light blue) powdery mildew infected Chardonnay grapes

investigations on the application of the MIR (mid-infrared) technique, used a rapid and simple sample presentation method called attenuated total reflectance (ATR). With this technique, the grape homogenate sample is simply smeared onto the ATR crystal and scanned, thereby eliminating many of the problems associated with the use of sample cuvettes, which require a very small pathlength in the MIR spectral range. Scanning of the homogenates of grape samples from the 2002 vintage was conducted on both the *NIRSystems 6500* (NIR) and an FT-MIR spectrometer. Comparative calibration statistics for the two techniques suggest that MIR may have superior predictive capability for pH and TSS, but is less accurate than NIR for colour. However, the best prediction for colour was achieved using a combination of MIR and NIR. Work is continuing with both of these techniques using samples from the 2003 vintage.

In late 2002, the project, together with the Institute's Analytical Service, commenced evaluation of an FTIR-based spectrometer (FOSS WineScan) for assessment of its capability in the routine analysis of wines. Wine samples, and corresponding analytical data, were used for re-calibration of the instrument, and the resulting calibrations used to enhance the 'global' calibrations provided with the instrument. Although the calibrations provided by FOSS allow analysis of up to 18 wine properties, the current study is examining only nine of these *viz.* alcohol, pH, density, glucose/fructose, total acidity, volatile acids, acetic acid, citric acid and malic acid. Preliminary results for the first six months of operation have been very encouraging and were presented in New Zealand in May 2002 at the FOSS Directions 2003 conference.

The project team has also been working with other groups in the application of NIR to predict red grape quality parameters (colour, TSS and pH) in relation to their research projects. The objective was to reduce the cost of analysis and the necessity for wet chemistry. Such work is underway with groups at CSIRO (Land & Water's Precision Agriculture group) and The University of Adelaide (Roseworthy Campus). Strengthening of relationships with wineries has resulted from both the development of some training material in chemometrics and multivariate analysis for wine industry personnel, as well as exchanging experiences through interaction with other industry staff working in the area of IR spectroscopy.
It is proposed that an 'IR spectroscopy users group' be established bringing together the Institute's NIR team, and other researchers and practitioners involved in the development and application of IR spectroscopy in the research and industrial communities. This will be an important step in further strengthening the links between the Institute and industry in the application of the IR technology in the Australian wine industry.

Grape composition and wine flavour

Staff: Dr Mark Sefton, Professor Peter Høj, Dr Leigh Francis, Dr Markus Herderich, Dr Elizabeth Waters, Dr George Skouroumounis, Dr Alan Pollnitz, Dimitra Capone, Mango Parker (nee Ranzijn), Kevin Pardon, Kate Lattey, Heather Smyth, Dr Gordon Elsey, Carolyn Puglisi, Aggie Janusz, Merran Daniel

This project has the long-term objective of understanding how the combination of viticultural and winemaking practices determine the aroma and flavour characteristics of wine and is divided into three broad areas: the identification of grape-derived wine components that affect wine aroma and flavour; studies of the formation and degradation of these wine flavour constituents including the identification of their grape-derived precursors; and finally, studies on how viticultural variables affect the production of wine flavour compounds and their precursors in the grape.

Analysis of wine components and their precursors

Analytical methods for 33 important fermentation esters, acids and alcohols using their deuterium labelled analogues as internal standards have been fully validated. Most of these compounds can be analysed in a single GC run. The methods are now being applied to other projects under this project *Grape composition and wine flavour* and *Yeast flavour and fermentation activity.*

A sample of deuterium-labelled citronellol has been prepared, and an analytical method for this monoterpene has been developed. The method is now being applied to sensory study of Riesling and Chardonnay wines (see below).

Large scale syntheses of the potent wine flavour compound 'wine lactone', its deuterated analogue, and the naturally occurring (optically active) form have recently been completed. These standards will be used to develop analytical methods for wine lactone in wines and in model media – the latter is needed for studies on the formation of wine lactone from grape-derived precursors. The optically active form will be used in the sensory studies of Riesling and Chardonnay (below).

Identification of new wine flavour components

Hydrolysates of a grape-derived glucoconjugate purified by MLCCC have been analysed by GC/olfactometry/mass spectrometry and these chromatograms compared with those of hydrolysates of the total glycoside pools isolated from grapes. The former are much simpler than the latter, and it has been possible to assign structures to five out of the six odorants detected in the chromatogram. Four of the odorants have previously been reported as wine components, and we have so far been unable to identify the fifth. The sixth component, which was also the most potent of the six in the GC/olfactometry assessments, was tentatively identified as 2,3,6-trimethylphenylbutadiene (TPB) from its mass spectrum. An authentic sample of TPB was synthesised and this has confirmed the assignment. TPB has not been previously reported as a grape or wine component. The threshold of TPB in a white wine was determined to be 40 ng/L and common descriptors used by our panel for this compound was 'floral', 'geranium' and 'tobacco'. This makes it one of the most potent wine flavour compounds known. An analytical method for TPB using d_8-naphthalene as internal standard was developed and several wine samples were analysed for TPB, which was observed in four out of the five white wines examined at a concentration of 50-210 ng/L. It was not observed in the four red wines examined. A more sensitive and accurate analytical method for TPB using stable isotope dilution analysis is well advanced – a deuterium labelled analogue has already been successfully synthesised in a multi-step pathway.

Formation of important wine flavour compounds from grape-derived precursors

Riesling Acetal is a relatively weak-smelling grape derived norisoprenoid that is formed along with the more potent 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) from grape derived precursors. In the short term, these two products are formed in competition, and we considered that factors such as the position of glycosylation on the polyfunctional precursors could steer the conversion towards or away from TDN formation. Recent hydrolytic studies by us have shown that Riesling Acetal can in fact be completely converted to TDN at wine pH, probably over a period of several years, and that this will contribute to a gradual increase in TDN in bottled wines over this period. As expected for acid catalysed transformations, the formation of TDN from Riesling Acetal was approximately twice as fast at pH 3.0 as at pH 3.2.

Four isomeric allenic diols, all potential damascenone precursors, have been synthesised and purified. The availability of these authentic samples allowed us to prove that they were intermediates in the formation of the important wine aroma compound damascenone, as had been postulated previously by us. Hydrolytic studies into the formation of damascenone have been conducted for all four isomers. At room temperature and pH 3.0, each isomer gave damascenone in high yield within a few days. Surprisingly, no trace of 3-hydroxydamascone was formed from these compounds. 3-Hydroxydamascone is normally found in grapes and wines at much higher concentration than is damascenone. These findings have led us to reevaluate the mechanism by which damascenone is formed in young wine.

Analysis by chiral gas chromatography of the intermediates formed in the hydrolytic studies has given us a valuable insight into the relative reactivity of the functional groups within the precursor molecule, and this has helped clarify our understanding of the formation of damascenone in nature.

A preliminary study concerning the reaction of damascenone with common wine components (i.e. the loss of damascenone as wines age) has been completed. Ten potentially reactive compounds were examined. The decrease in damascenone concentration in these reactions was less than expected, and alone cannot account for the more rapid decrease in damascenone concentration normally seen in ageing wine. The expected products of these reactions were synthesised, and one of these has been identified as a minor byproduct of damascenone formation from the allenic precursors discussed above.

The synthesis of a group of grape-derived thiols that are key flavour impact compounds in many red and white wine styles is in progress. Two of the three target compounds have been prepared, and a sample of a cysteine conjugate of one of these has also been synthesised. This conjugate is now being used in the *Yeast flavour and fermentation activity* research program on flavour release during fermentation (cysteine conjugates are grape-derived precursors of these important thiols). A synthesis of deuterium labelled analogues is also in progress. These analogues will enable us to develop stable isotope dilution assays for these compounds in grapes and wines.

A study into the formation of sulfur analogues of common grape components is underway. This work is being done to see if these compounds are important contributors to wine aroma and flavour. At present, three such compounds have been synthesised, with a fourth currently under investigation. One of these compounds has a very low aroma threshold. A method to determine its concentration in wine will be investigated in the near future.

Relationship between composition and sensory properties of wine

A sensory descriptive analysis study on a set of 20 commercial Riesling wines and 20 one to two-year old commercial unwooded Chardonnay wines has been completed. The sensory data indicate that, for the Riesling wines, there were three main groups of samples: those young wines which were dominated by floral and lemon aromas, those that were tropical fruit/passionfruit-like, and older wines with lime, kerosene and toasty attributes. Within each broad group there were wines with varied intensities of specific attributes, such as samples with a relatively strong floral perfumed aroma compared to those with a dried rose character, and wines with stronger honey-like aroma. For the Chardonnay wines, honey, butterscotch, woody and spicy descriptors were prominent in the two-year old wines, while the one-year old wines were dominated by estery, citrus and fruity notes. A subset of these younger wines also showed prominent passionfruit and herbaceous characters.

The data set should enable insights to be made into the relationships between the concentration of particular volatile compounds and the intensity of Riesling and Chardonnay wine flavour attributes once all chemical analyses have been completed. For the Riesling wines, the concentration of esters, alcohols and acids have already been quantified, as well as the major monoterpenes and nor-isoprenoids. The compositional analyses of all of these wines will be largely completed in the near future, and some preliminary data analysis has already been undertaken.


Gordon Elsey & Heather Smyth

The influence of oak cooperage on wine composition

Staff: Dr Mark Sefton, Dr Alan Pollnitz, Dimitra Capone, Kerry Wilkinson, Kevin Pardon

All four possible isomers of the glucosides of *cis*-and *trans*-oak lactone have been synthesised and obtained in pure form. These compounds are considered to be potential precursors of oak lactone and are structurally similar to a galloylated glucoside already identified in oakwood. The simple glucosides, therefore, serve as useful models to study the reactivity of the galloylated analogue.

The glucosides were relatively stable at wine pH, and even at 100˚C, reacted only slowly to give oak lactone. Both these glucosides and the galloylated derivative would not, therefore, be expected to hydrolyse to oak lactone during wine conservation, unless active glycosidase enzymes (or microorganisms with glycosidase activity) were present in the wine. Such compounds cannot account for the additional amounts of oak lactone that can continue to be generated in model wine extracts of oak shavings by hydrolytic means, once the shavings have been separated from the extract.

When the glucosides of *cis*- and *trans*-oak lactone were absorbed onto oak shavings and the shavings were then heated to 235˚C in an oven, substantial increases in the amount of oak lactones in the shavings were observed. This demonstrates that the simple glucosides and probably also the galloylated glucoside can act as precursors to oak lactone during barrel toasting. Increases in oak lactone in oak as a result of heating have been reported by many authors.

As a result of our obtaining pure isomers of the glucosides of the oak lactones, we have been able to resolve the nature-identical isomer of *cis*-oak lactone from its non-natural optical isomer. Previous sensory work on *cis*-oak lactone in wine has been carried out on mixtures of these two forms (i.e. the racemate), or even on mixtures of *cis*- and *trans*-oak lactone.

The sensory detection threshold of the nature-identical isomer of *cis*-oak lactone (which is ten times more potent than the *trans* form) in a neutral dry white wine was 23 µg/L. This is much lower than the value of 90 g/L previously reported for the racemic mixture. Some individual panellists were able to detect the natural isomer at a concentration below 10 µg/L. The same sensory panel obtained a detection threshold for the non-natural isomer of 86 µg/L. Not only did these two isomers differ in their odour intensity, but informal comments by the sensory panellists indicated that the aroma descriptors for these isomers were also different. We are now in a position to

carry out formal sensory descriptive analysis on wines spiked with the nature-identical isomer of *cis*-oak lactone in order to determine its sensory properties more rigorously.

A previous report by American researchers hypothesised that *cis*- and *trans*-oak lactones occur in equilibrium with their open chain forms, and that conflicting data on oak lactones in oakwood in the literature was a result of the failure to take such equilibria into account. We have, therefore, determined the acidity constants (pKa) of the open chain forms, their rates of lactonisation at different pH values and ethanol concentrations, and the extent of lactonisation under these various conditions. The data show that, at wine pH (3.0 – 3.8), oak lactones exist almost entirely in the lactone form at equilibrium, in contrast to the earlier hypothesis. At the highest pHs studied, however, the open chain form of the *cis*-isomer lactonised relatively slowly. At pH 3.8, 50% lactonised after 16 days at room temperature, and at pH 5.0 in model spirit, only a few per cent lactonised after several days. The *trans* isomer lactonised some 14 times faster than the *cis* isomer under all conditions. As it is possible some oak lactone may originally be formed in wood in open chain form, these data indicate the need for suitable soaking times and low pHs of wine or aqueous extracts when preparing extracts of oak shavings for analysis.

Chemical analysis of industry technical problems

Staff: Dr Mark Sefton, Yoji Hayasaka, Gayle Baldock, Dr Alan Pollnitz, Dr Markus Herderich, Tracey Siebert, Dimitra Capone, Kevin Pardon

Problem solving work and research into the formation of 4-ethylphenol and 4-ethylguaiacol in wines is carried out by the wine chemistry team in collaboration with, and under the direction of, the Industry Services section. Accordingly, the results of this work are given in the report by Industry Services. A main role of the project *Chemical analysis of industry technical problems* is to provide analytical support to these areas.

Analytical methods for taint compounds in wine continue to be developed. We have fully validated methods for compounds derived from petroleum products, including toluene, styrene, C2 alkyl benzenes (e.g. xylenes), C3 alkyl benzenes (e.g. trimethylbenzenes), C4 alkyl benzenes (e.g. tetramethylbenzenes), naphthalene, methylnaphthalenes, dimethylnaphthalenes and trimethylnaphthalenes. A method for 2-aminoacetophenone (reported to be responsible for the so-called 'untypical ageing' taint encountered in some German white wines) using stable isotope dilution analysis (SIDA) has also been validated and is being used to survey Australian wines. A series of deuterium-labelled chlorophenols have been synthesised and are being used to develop methods for their unlabelled analogues. These methods are well advanced. In the area of *Brettanomyces* research we have fully validated SIDA methods for 4-ethylguaiacol, 4-vinylguaiacol, 4-vinylphenol and isovaleric acid. These methods supercede less reliable and more time-consuming methods previously used.

Yeast flavour and fermentation activity

Staff: Dr Paul Henschke, Jeff Eglinton, Dr Eveline Bartowsky, Dr Peter Costello, Jane McCarthy, Kate Howell, Professor Peter Høj, Dr Mark Sefton, Dr Alan Pollnitz, Tracey Siebert, Dimitra Capone, Gayle Baldock, Dr Diego Torrea

This project has the long term aim to understand the role that yeast have in modulating wine sensory characteristics so that winemakers can more effectively exploit the benefits of yeast for commercial gain. During the past year, work has focussed on model fermentation studies related to understanding the role of yeast in development of red wine colour, commercial trials with experimental *Saccharomyces bayanus* strains, and applying the stable isotope dilution assay suite to experimental Chardonnay wines to understand better how fermentation conditions affect aroma profile.
Dr Diego Torrea, a recent PhD graduate from Professor Carmen Ancin's laboratory, Navarro University, Pamplona, Spain, has been awarded a two-year fully funded post-doctoral scholarship from the Spanish Government to work on aspects of the role of yeast in development of wine aroma. Diego already has considerable experience on chemical analysis of wine aroma and collaboration with Professor Ancin's team will greatly benefit this project. Dr Peter Costello has resigned from the Institute (July 2003) to take up a position as the National Technical Manager with Lallemand Australia. Peter brought a wealth of research and industry experience to this project and contributed strongly to chemical analysis as well as fermentation microbiology. As in previous years, this project has benefitted from enthusiastic collaboration by a small group of winemakers in trialling experimental Institute yeast and providing wines and technical information. This close association is important in maintaining a commercial focus on project work and maximising the benefit of limited resources.

Saccharomyces bayanus – a research tool for understanding the role of yeast in modulating wine sensory characteristics and a fermentation yeast with commercial potential

Saccharomyces bayanus is a species of the *Saccharomyces sensu stricto* group together with the globally favoured wine yeast, *S. cerevisiae*. The consequential sharing of many qualitatively similar physiological and biochemical attributes, facilitates deployment of *S. bayanus* in wineries with familiar *S. cerevisiae* technology. In addition to being a cryotolerant yeast, that is, having a lower temperature range for growth and fermentation, our research, and that of others, is showing that *S. bayanus* has other properties of oenological interest. These properties are being investigated both in the laboratory and by collaboration with winemakers working in the commercial environment. The laboratory-based work has the aim to identify oenological differences with *S. cerevisiae,* the universal reference yeast, and to gain an understanding of their physiological and biochemical basis so that winemakers can benefit. By working closely with winemakers, we can develop a better understanding of the commercial potential of various yeast properties, and to focus our research effort more closely. Progress, which has also benefitted greatly from collaboration with the Wine flavour, Polymers and Sensory teams in the Institute, has been summarised in previous annual reports.

Our work is currently focussed on the volatile and non-volatile metabolites of *S. bayanus* that have potential winemaking benefits. Higher amounts of glycerol and lower amounts of acetic acid (a small reduction in ethanol is also observed) together with increased succinic acid levels are typically formed at usual winemaking temperatures (Institute publications #632 and 647). *S. bayanus* is typically competitive and dominates fermentation (Institute publication #707) and although it may exhibit a slower rate of fermentation towards the end of fermentation, the wines generally have low residual sugar. The prolonged fermentation times may be linked to improved palate structure and weight noted by a high proportion of industry trials. Work is currently in progress to define better this important wine attribute and to establish its chemical basis.

In white wines, the fruity aroma of *S. bayanus* is often described as being more complex than that achieved with *S. cerevisiae* (Institute publications #632, 647, 679 and 693). For example, Chardonnay wines made with *S. bayanus* are typically rated higher in the attributes *cooked orange peel, botrytis/apricot, yeasty, savoury, nutty* and *aldehyde* and lower in the *estery, pineapple, peach* and *citrus* attributes compared with those wines made with *S. cerevisiae*. Commercial Chardonnay wines have shown some *rose* aromas which are characteristic of the compounds, 2-phenylethanol and 2-phenylethyl acetate, with much less obvious *aldehyde-like* or *oxidised* aromas. *S. bayanus* appears to produce a different profile of fermentation esters and alcohols, and work is in progress to identify the key impact compounds associated with *S. bayanus* wine aroma and to establish how winemaking conditions affect them. This work is benefitting from the suite of stable isotope dilution assays recently developed by the Institute's Wine flavour team led by Dr Mark Sefton (see previous annual report).

Saccharomyces bayanus appears to have red winemaking potential with respect to aroma, palate and colour. The limited trials undertaken so far suggest that Cabernet Sauvignon wine made with *S. cerevisiae* AWRI 838 exhibited brighter purple colour and fresh fruit aromas and flavours, and wine made with *S. bayanus* AWRI 1375 showed leaner, slightly lighter fruit with more complex aromas and flavours. The chemical basis for these aroma differences is being investigated with the stable isotope dilution assays as is being done for the *S. bayanus* white wines.

The observed difference in red wine colour resulting from the use of *S. bayanus* has been supported by analytical measurements as reported in the previous annual report, presented at a recent international conference (Eglinton et al. 2003, ACS proceedings, in preparation) and recently published in an industry journal (Institute publication #729). This important winemaking attribute, which has its basis in wine phenolics, is the subject of a large research effort by the Institute's Tannin and Wine Microbiology teams. Aspects of this work related to the role of yeast in determining wine colour are reported below.

Probing the role of yeast in the development of red wine colour - model fermentation studies

The results of studies showing that some yeast species and strains have the ability to modify the colour density and/or hue of red wine have been summarised in previous annual reports. For example, a major difference between Cabernet Sauvignon wines made with *S. cerevisiae* or *S. bayanus* was the higher concentration of 'pigmented polymers' in the *S. bayanus* wines (Institute publication #729). The basis for the development of 'pigmented polymers' during fermentation has been investigated using a model fermentation system in the laboratory, and *S. cerevisiae* in the first instance. The model system consisted of a synthetic medium that was supplemented with different combinations of tannins, anthocyanins, and yeast cells.

'Pigmented polymers' were formed in model solutions containing anthocyanins and/or tannins and yeast. The formation of 'pigmented polymers' was accompanied by a decrease in the concentration of the anthocyanins, suggesting that some of the anthocyanins were being converted into 'pigmented polymers.' Despite some 'chemical' formation of 'pigmented polymers' in the absence of yeast cells, actively fermenting yeast cells were required for maximal formation of 'pigmented polymers.'

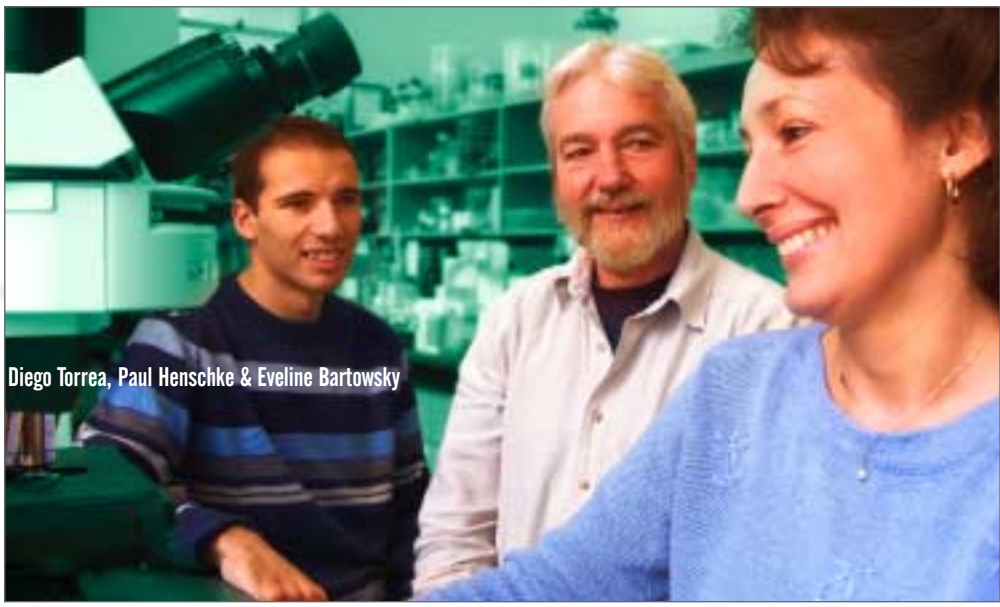
This study demonstrated the usefulness of the model system for the study of yeast-mediated reactions involving the formation of red wine pigments. A draft manuscript has been prepared to disseminate this information to industry. Some aspects of the study have been presented at an international conference (American Chemical Society, USA – See Appendix 1) by Dr Markus Herderich and incorporated in the manuscript for the conference proceedings. This work continues as a collaboration between the Wine Microbiology and Tannin teams under the supervision of Jeff Eglinton and Dr Markus Herderich.

Winery experience with Saccharomyces bayanus fermentation strains

Considerable interest is being shown by winemakers in the use of two of the Institute's *S. bayanus* strains, AWRI 1176 and 1375. Some ten companies in Australia and New Zealand have conducted winery trials ranging from barrel to 10 hL ferments since 1999. Findings arising from several of these trials have been communicated to winemakers in industry journals and via Institute Roadshow seminars and the *Winemaking with non-conventional yeasts* workshop conducted at the 11th Australian Wine Industry Technical Conference in October 2001. Over recent vintages, more extensive trialling by two companies has led to wine being used in commercial blends. The trials have focussed predominantly on Chardonnay, but have included Semillon, Sauvignon Blanc, Pinot Noir and Cabernet Sauvignon.

In the early white wine trials, winemakers typically noted a greater diversity of aroma notes, such as *cooked orange peel, botrytis/apricot, yeasty, savoury, nutty* and *aldehyde,* in addition to a lowered impact of the fruity aromas typically associated with *S. cerevisiae* strains. Occasionally, more complex aroma notes were observed. In general, wine aroma is distinctive with a tendency to *cooked fruits/old rose/savory/nutty* notes while retaining fruitiness and good expression of varietal character. More recent trials are focussing on palate structure and weight. Winemakers consistently report palate fullness and richness, and often a creamier texture, imparted by *S. bayanus* in comparison with *S. cerevisiae*. In barrel trials, better oak integration is commonly noted. Many of the winemakers involved with these trials see *S. bayanus* as providing an additional blending option for the purpose of building wine style.

Winery trials with red varieties have been constrained by the lack of commercial starter culture availability. This issue is currently being addressed as reported below.



Diego Torrea, Paul Henschke & Eveline Bartowsky

Saccharomyces bayanus starter culture development

Initial interest in the Institute's *S. bayanus* strains was confined mainly to barrel trials for which only small volume starter cultures are needed. However, the more extensive winemaking trials planned by several companies is being restricted by availability of suitable starter cultures and the time/resources required to culture them from a laboratory source. Currently, the Institute's *S. bayanus* strains are only available as agar slope cultures, and requires experienced staff and suitable facilities to propagate these yeast to commercial quantities. Use of the strains by industry could be expanded if these yeast were available as active dried wine yeast (ADWY). The Institute has begun a collaborative project with Lallemand Inc, a major international yeast manufacturer and a financial supporter of selected Institute research, to investigate the potential to produce ADWY from AWRI 1176 and 1375. The outcome of a successful conclusion to this project will be the ready availability of these strains in a commercially useful form.

Isolation of Saccharomyces bayanus winemaking strains

Numerous studies at the Institute over recent years have demonstrated the potential for the use of *S. bayanus* strains to modulate the physical, chemical and sensory profiles of white and red wine (Institute publications #632, 647, 679, 693, 707 and 729). These projects and extensive trialling by commercial wineries across Australian wine producing regions since 1999 have depended largely on two strains of *S. bayanus*, AWRI 1176 and 1375, isolated at the Institute. It has been unknown, however, if these strains are representative of the *S. bayanus* species, or if other isolates with better winemaking properties can be found. We can draw an analogy with *S. cerevisiae* strains, in which the extent of winemaking properties of that species was not well appreciated until the landmark work carried out at the Institute by Dr Bryce Rankine and colleagues in the 1960s and 1970s (Institute publications #55 and 156); strains AWRI 350, AWRI 729 and AWRI 796, remain widely used globally for wine production.

Twelve strains of *S. bayanus* have now been collected, with most strains being isolated from samples collected from different regions of Australia. The taxonomic identity of the strains was confirmed by molecular techniques. In collaboration with Dr Miguel de Barros Lopes, Jeff Eglinton and Jenny Bellon, the genetic relatedness of these strains has been determined by Amplified Fragment Length Polymorphism (AFLP) analysis (Institute publication #589). The strains appear to cluster into three groups: one including the strains 1176, Sb3 and Sb4; one including 1375, 1438, 1439 and 1443; and one including the remaining strains (Figure 5). Interestingly, the *S. bayanus* isolates appear to be more closely related to the type strain of *S. uvarum* (a yeast that is now classified as *S. bayanus*) than they are to the type strain of *S. bayanus.* This observation suggests that the genus *S. bayanus* may harbour a wide diversity of potentially useful phenotypes. Other *Saccharomyces sensu stricto* species, such as *cerevisiae* and *paradoxus,* are included in the analysis for comparison.

This result is interesting because the strains, AWRI 1176 and 1375, have been well characterised at the Institute. Wines produced using these strains can be quite different from a sensory point of view, and the yeast strains appear to be separated by AFLP analysis. Future phenotypic investigations will be conducted on these new *S. bayanus* isolates, but this analysis is time- and labour-intensive. If AFLP analysis can be used to predict, in part, the phenotype of the strains, then it could represent an adjunct to phenotypic studies that would result in more efficient characterisation of future yeast isolates.

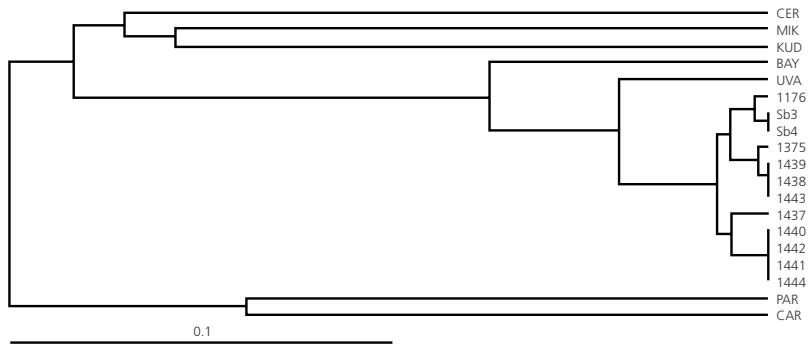


Figure 5. Phylogenetic tree (one statistically possible outcome) showing the relatedness of different *Saccharomyces* species and *S. bayanus* isolates. Type strains: CER, *S. cerevisiae*; MIK, *S. mikatae*; KUD, *S. kudriavzevii*; BAY, *S. bayanus*; PAR, *S. paradoxus*; CAR, *S. cariocanus*; UVA, *S. uvarum*. 1176, 1375, Sb3, Sb4 and 1437-1444 are isolates of *S. bayanus*.

Systematic studies to establish the role of yeast and fermentation conditions on modulating the yeast derived volatiles of wine

The compounds that define wine aroma can be divided into essentially two groups on the basis of their origin: the grape berry secondary metabolites which are largely responsible for varietal definition; and the products of yeast metabolism which provide the vinous character of wine, and is often referred to as the 'fermentation bouquet'. The major yeast volatile metabolites, which comprise esters, alcohols, carbonyls and fatty acids, are derived from sugar and amino acids metabolism. Many factors are known to influence the quantitative profile of yeast volatile compounds, and include the species and strain of yeast, the concentration of nutrients, sugar, amino acids, oxygen and lipids, and physico-chemical conditions, pH, temperature, agitation, solids, and so on. Surprisingly, although much has been published, little systematic information is available on the relative importance of these factors, and consequently winemakers rely heavily on empirically-derived detail.

The recent development of a suite of stable isotope dilution assays for quantifying some 30 yeast volatile compounds by Dr Mark Sefton's team provides the opportunity to precisely establish the relative importance of yeast species/strain and fermentation conditions on the development of wine aroma. Association of chemical data with quantitative descriptive sensory analysis will allow identification of key impact aroma compounds which can ultimately be used to predict better the sensory profile of wine from chemical data. Such knowledge would assist winemakers in 'steering' wine style.

Dr Diego Torrea has commenced studies on establishing a laboratory method and experimental conditions. A Chardonnay grape juice has been selected, the chemical profile determined and a chemically defined grape juice medium formulated to mimic, as far as practical, the composition of the grape juice. The grape juice and model medium have been fermented in the presence and absence of a lipid supplement, since the lipid content (determined by the level of solids in grape juice) is known to profoundly affect yeast fermentation performance and available evidence suggests that lipids also affect the aroma profile of wine. The ferments were also conducted in the presence and absence of oxygen, another critical yeast nutrient that affects yeast fermentation performance and wine aroma profile. The use of oxygen during fermentation has been promoted by some researchers but little information regarding impact on wine aroma profile has been published. Once the important lipid and oxygen parameters have been defined in terms of the volatiles profile, work will focus on determining which amino acids are important in determining the aroma profile, since this nutrient source has been found to be highly variable in Australian juices and musts and has a considerable effect on fermentation rate, residual sugar and the formation of undesirable sulfide.

Preliminary analysis of the results concerning the presence or absence of oxygen and lipids on the volatiles profile of a Chardonnay wine has revealed a complex pattern with respect to esters, alcohols and fatty acids. Each of these groups of aroma compounds responds in a different manner suggesting that the choice of conditions will have an important impact on wine style. This work will shed light on the relative importance of these conditions.

Malolactic fermentation and wine flavour

Staff: Dr Paul Henschke, Dr Eveline Bartowsky, Dr Peter Costello, Jane McCarthy, Melissa Fettke

This project has the long term aim of defining and understanding the role that malolactic fermentation has on modulating the sensory characteristics of wine. A secondary aim is to investigate the basis for unreliable induction of this secondary fermentation process. The outcomes of this research will allow winemakers to more efficiently control and exploit MLF for guiding wine style to enhance commercial benefit.

Studies over the past year have focussed on wine yeast and malolactic bacteria physiological compatibility, developing and using molecular tools for understanding the ecology of malolactic bacteria, and defining the role of the flavour compound, diacetyl, and controlling its presence, in wine.

Recent contributions have centred on the bacterial origin of the mousy off-flavour N-heterocycles and establishing the importance and nature of yeast and bacteria interactions as it impacts on the MLF.

Physiological interaction between wine yeast and malolactic bacteria – the role of amino acids in affecting compatibility

Despite recent developments in strain selection and starter culture preparation, induction and completion of malolactic fermentation (MLF) remains an unreliable process which is applied to most red wines and a proportion of non-aromatic white wines. In addition to important physico-chemical and nutritional properties of wine, there is growing awareness of the potential interactive effects wine yeast can have on the growth and metabolism of wine malolactic bacteria (reviewed by Alexandre, Costello, et al. 2003 – submitted for publication). Such physiological interactions have been shown to inhibit or stimulate the growth of malolactic bacteria, and are considered to arise from competition for essential nutrients, and the production of metabolites that are bioactive towards malolactic bacteria (Gockowiak and Henschke 2003, *Australian Journal of Grape and Wine Research,* accepted for publication). From a nutritional perspective, the uptake and release of amino acids by yeast, and release of more complex yeast-derived nitrogenous compounds, including peptides and mannoproteins, during and after alcoholic fermentation may have significant impact on the growth and metabolism of malolactic bacteria. In this regard, the current study aimed to systematically investigate whether changes in the content of amino acids and other nutrients during alcoholic fermentation and after extended yeast lees contact can influence the growth response of malolactic bacteria in a chemically-defined model system.The methodology has been published recently (Institute publication #732).

In this study, a chemically-defined grape juice medium, previously formulated for the study of yeast-bacteria interactions, was fermented to dryness with three commercial *Saccharomyces cerevisiae* wine yeast strains, selected on the basis of the results of an extensive survey of yeast-bacteria interactions reported in the Institute's 2002 Annual Report. The medium was formulated to contain all the principal yeast nutrients at non-growth limiting concentration, in particular all amino acids were supplied to excess. Sub-lots of each test wine were then clarified either immediately after fermentation, or after contact with yeast lees for two weeks with regular stirring. The resultant wines were then tested under standardised conditions (pH 3.5, 10% v/v ethanol, negligible SO_2 content) for bacterial growth response. In order to determine whether the yeast had depleted essential nutrients needed for ML bacterial growth, the wines were treated by supplementation with amino acids and/or other nutrients selected from the chemically-defined grape juice medium. Wine that received no supplements served as the reference wine. The treated and reference test wines were then inoculated with four different strains of *Oenococcus oeni* to test for strain-specific growth responses. Bacterial growth response was monitored over a five-week period.

Under the conditions of this study, the results show that bacterial growth and MLF in fermented test wines was generally not limited by the yeast mediated depletion of amino acids and/or other nutrients that compose the chemically defined medium. The level of bacterial growth response obtained corresponded to a 'compatible' yeast-bacteria interaction, typical of that which would occur when no nutrients have been depleted to a growth-limiting concentration. However, for two of the three yeast strains, the overall proliferation of bacteria was greatly stimulated by test wines that had received yeast lees contact. This lees-induced stimulation of bacterial growth was not associated with an increase in amino acid content. Rather, during the production of the latter test wines by fermentation, yeast viability was observed to significantly decline during the lees contact period, and the concentration of peptides greatly increased.

One possible interpretation of these results is that enhanced proliferation of bacteria in wine made by contact with yeast lees is associated with a yeast strain-dependent autolytic release of cellular products, and offers the possibility that peptides might stimulate growth of bacteria. Such a hypothesis, however, will require rigorous testing to establish its validity.

This work was performed by Ms Melissa Fettke at the Institute during honours studies for her BAgSc degree at The University of Adelaide, for which she was awarded a first class honours degree. Drs Peter Costello, Eveline Bartowsky and Paul Henschke supervised this project. Melissa undertook vintage experience with Rosemount in the McLaren Vale.

Genetic similarity and strain differentiation of commercial and wine isolates of the malolactic bacterium Oenococcus oeni

Comparative analysis of the genome of bacterial strains provides the information to determine intraspecific variation or genetic similarity of strains, and provides a basis for strain differentiation. At the applied level, it is usually sufficient to generate a simple DNA fingerprint of each genome using conventional polymerase chain reaction (PCR) technology, as summarised in the Institute's 2002 Annual Report. The fingerprinting technique, randomly amplified polymorphic DNA (RAPD), which was previously applied to *Acetobacter pasteurianus* strains that were associated with random microbial oxidative spoilage of bottled red wine (Institute publication #718), was successfully applied to the malolactic bacterium, *Oenococcus oeni.* The RAPD methodology to unequivocally differentiate *O. oeni* strains of commercial and wine origin has been published recently (Institute publication #733).

Commercial preparations of *O. oeni* that are widely used for the induction of MLF in red and white wines by Australian wineries have been isolated mainly from European wineries. Genetic similarity amongst several of these cultures was examined using the RAPD technique. As shown in Figure 6, the four commercial cultures are genetically distinct. Interestingly, Lalvin 41, which is genetically most dissimilar, is claimed by Lallemand to be especially tolerant of several adverse wine conditions. Rigorous studies would be needed to test for association between RAPD markers and physiological properties, which, if proved to be the case, could assist in the development of strains with improved winemaking properties.

In addition, we also examined the genetic similarity between Australian *O. oeni* isolates and compared these with the commercial cultures. These comparisons are also depicted in Figure 6. The Australian winery isolates were obtained from wines undergoing uninoculated (spontaneous) MLF, and from a yeast trial conducted on Cabernet Sauvignon wines during the 2002 vintage in the

Hickinbotham Roseworthy Wine Science Laboratory (WSL). A preliminary analysis of the genetic relatedness of strains was given in the Institute's 2002 Annual Report.

The winery isolates are mostly genetically distinct and the intraspecific variability across Australian wineries is at least as great as that represented by the four commercial European strains. Interestingly, several isolates from three wineries (Wineries I, V and IV, respectively) are closely related to several commercial cultures (Lalvin EQ54, Viniflora oenos and Lalvin 31) suggesting that these isolates may be derived from commercial cultures, several of which have been used in Australia for five to ten years. The genetic variability of *O. oeni* in Australian wineries suggests that these indigenous strains possess a wide range of physiological and biochemical properties which could be expected to reflect the wide diversity of wine environments.

Malolactic fermentation and wine flavour – diacetyl and the 'buttery' attribute, a precursor investigation

One of the main volatile flavour components attributed to MLF activity is diacetyl (2, 3-butandione). Diacetyl confers a 'buttery' attribute to the wine, which, depending upon the concentration and wine style, is perceived as desirable or an off-flavour. Through a survey of 93 commercial bottled wines, we have recently shown that the perception of diacetyl is not only dependent upon its concentration, but also on the presence of other wine aromatic components, including fruitiness, aged aromas and dominant oak-like aromas (Institute publications #689 and 709). Figure 7 illustrates this point for Australian Chardonnay and Shiraz wines by comparing diacetyl concentration with the 'buttery' aroma score as determined by a trained sensory panel. In this survey, the diacetyl concentration was close to its published sensory threshold value for Chardonnay wines and below the sensory threshold value for Shiraz wines, suggesting that Australian winemakers currently favour a wine style having a low 'buttery' impact. Diacetyl concentration was also observed to decline with bottle age for the set of red wines studied, which would further reduce the 'buttery' character.

Because of the biological origin and chemically reactive nature of diacetyl, especially its ability to form an adduct with sulfite and its susceptibility to bio-reduction to the diol, there are numerous winemaking practices that can be used to modulate the desired concentration and sensory impact of diacetyl in wine. These practices, which have been published (Institute publications #637 and 689) are summarised in Table 3. Important factors include bacterial strain, inoculation rate, wine chemical composition (in particular, concentration of citric acid which is the bacterial substrate for diacetyl), lees contact and timing of wine stabilisation with sulfur dioxide. A review article on diacetyl and wine flavour has been prepared for publication, and signals the completion phase of this topic as a precursor study for developing techniques to study the relationship between bacterial metabolism and wine flavour.

Microbiological analysis of industry technical problems

Staff: Dr Paul Henschke, Dr Eveline Bartowsky, Dr Peter Costello, Jane McCarthy, Peter Graves

This project undertakes to investigate specific winemaking microbiological problems of importance to the wider industry. Recent topics have included mousy-off flavour occurrence and formation in wine, *Dekkera/Brettanomyces* spoilage of wine, and wine microbiological spoilage workshop as summarised in the previous annual report. Work has continued on the *Dekkera/Brettanomyces* spoilage project and on a case study concerning microbial mediated oxidative spoilage of bottled red wine. Small studies concerning application of lysozyme in winemaking, yeast spoilage of sweet wine and alcohol yield of fermentation yeast were also undertaken. Project AWR 5 also provides resources for maintaining the Institute's culture collection which provides yeast and bacteria for teaching and research, performing advanced wine microbiological analysis, undertaking dried yeast quality testing, and providing microbiological consultation to industry personnel.

Table 3. Suggested guidelines to manipulate the diacetyl content and 'buttery' flavour of wine using malolactic fermentation

Diacetyl content	Winemaking manipulation
High	Bacterial strain with high potential for diacetyl production Lower inoculation level Increase redox potential Avoid contact with yeast lees Stabilise wine immediately after malic and citric acid degradation
Low	Bacterial strain with low potential for diacetyl production Higher inoculation level Prolonged contact with yeast and bacteria lees before stabilising wine

Dekkera/Brettanomyces – Isolation from wine and a preliminary physiological characterisation

As forecast in the Institute's 2002 Annual Report, several of the aims of the Institute's multidisciplinary collaborative *Dekkera/Brettanomyces* project, coordinated by Peter Godden through the GWRDC-funded project *Evaluation of new analytical techniques and of processing aids for winemaking,* was to acquire a knowledge base of published work, isolate and characterise strains from Australian wines, and ultimately to provide information to winemakers for practical control of this common spoilage yeast. Readers are referred to progress reports for projects *Evaluation of new analytical techniques and of processing aids for winemaking* and *Selection and improvement of wine yeasts by application of molecular biology* for additional reports on current progress.

A review of the published literature on *Dekkera/Brettanomyces* shows that its classical physiological properties of relevance to alcoholic beverages (nutrient requirements, tolerance to pH, temperature, alcohol, sulfite, etc.) have been, in broad terms, described. However, little attention has been given to the strain variability of these properties. The molecular characterisation of Australian isolates in progress at the Institute (see progress report for project *Selection and improvement of wine yeasts by application of molecular biology*) suggests a wide genetic diversity of strains, and therefore the possibility of high variability in physiological and biochemical properties of relevance to wine production. Therefore, our focus will be on the characterisation of Australian isolates as opposed to culture collection strains that form the basis of much of the published work. For example, strain variability with respect to important wine parameters seems to be underestimated. In common with *Saccharomyces* yeast, *Dekkera/Brettanomyces* yeast appear to have very simple nutrient requirements suggesting that this feature could, under some conditions, offer a means for control. For example, it has been established by Chatonnet's group in Bordeaux, that lowered residual sugar content of wine can be used to limit growth and volatile phenol formation. Comparative physiological characterisation of Australian isolates can be expected to point to additional control measures.

The origin of Dekkera/Brettanomyces yeast in the winery is still unclear, and may include infected wine, poor sanitation of equipment and the common fruit fly. A recent publication (Connell et al. 2002 AJEV 53:322-324; 2002) suggests that this yeast can be spread through a winery by air currents. The physical environment of a winery may also prove to be a risk factor in Dekkera/Brettanomyces contamination and control. The molecular characterisation of Australian isolates in progress at the Institute will

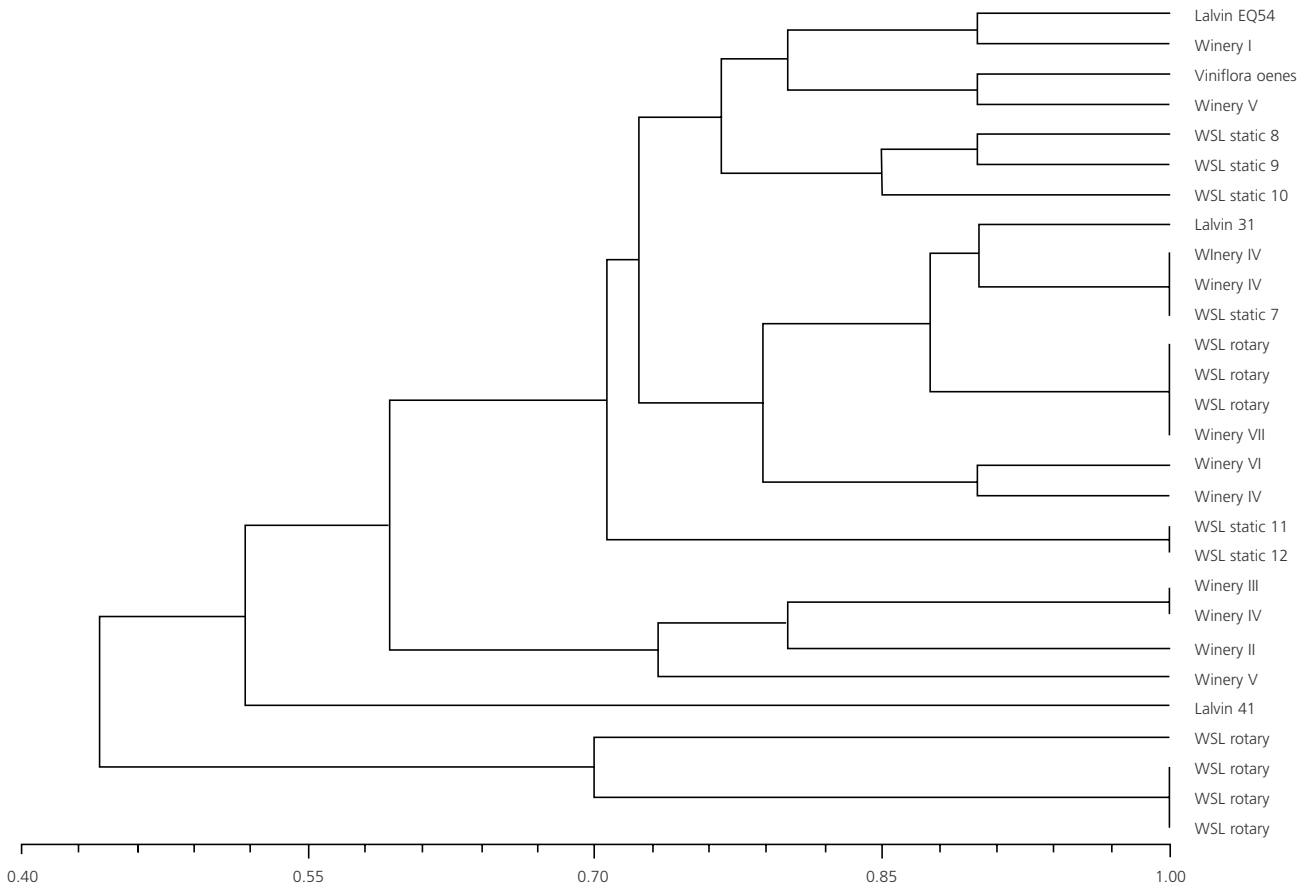


Figure 6. Cluster analysis of *Oenococcus oeni* strains isolated from different Australian wineries (I to VII), commercial cultures and a Cabernet Sauvignon yeast trial conducted in the Hickinbotham Roseworthy Wine Science Laboratory (WSL) during 2002 vintage.

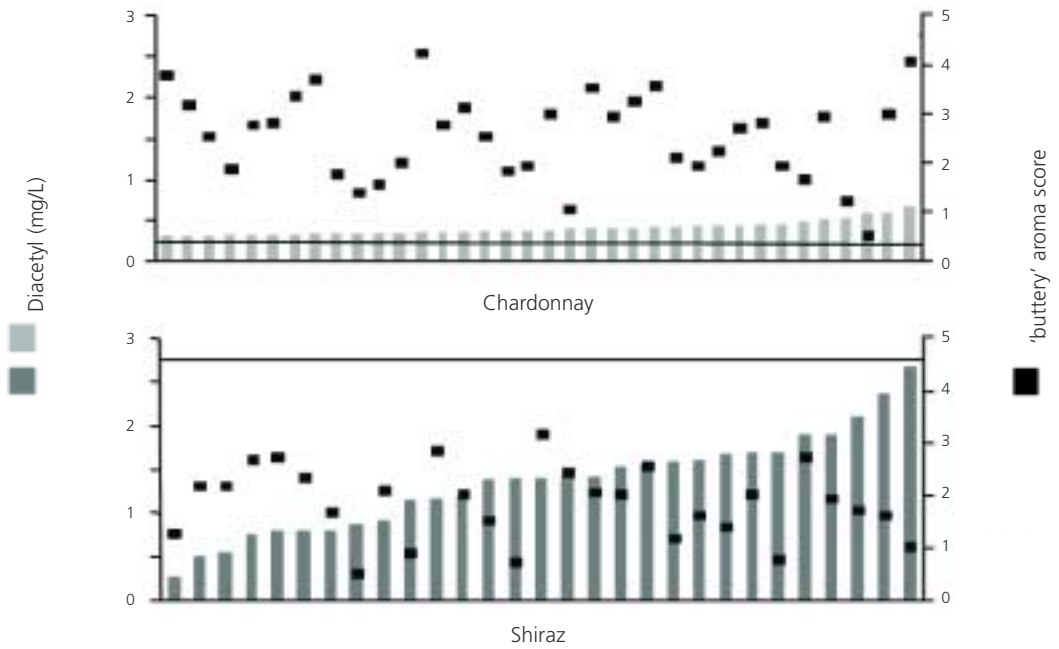


Figure 7. The diacetyl concentration and 'buttery' sensory perception of 36 Australian bottled Chardonnay and 29 Shiraz wines. The horizontal lines marking a diacetyl concentration of 0.2 mg/L for Chardonnay and 2.8 mg/L for Shiraz wines indicate the respective reported sensory thresholds for diacetyl. The panel was screened for their ability to detect diacetyl in wine and were involved in numerous training sessions where their performance was assessed.

also contribute to this question (see progress report for project Selection and improvement of wine yeasts by application of molecular biology).

Dekkera/Brettanomyces yeast generally proliferate only slowly in wine and cell numbers are often very low, necessitating efficient recovery. Classical methods rely on the use of elective media to target Dekkera/Brettanomyces yeast followed by simple test procedures that provide a presumptive identification. They have morphological, physiological and biochemical characteristics that assist their selective isolation from wine: ogival and profusely elongate cells, slow colony growth, acid production, tolerance to cycloheximide and growth on L-lysine and ethanol. A survey of various media has confirmed that the recently developed Differential Dekkera/Brettanomyces Medium published by Louriero and colleagues (J. Appl. Microbiol. 90:588-599; 2001) is more effective than conventional media supplemented with the fungicide cycloheximide, although this medium still supports the growth of several other yeast species. Identification is confirmed by a DNA (RFLP) method (see progress report for project Selection and improvement of wine yeasts by application of molecular biology).

A related issue seems to be the difficulty of recovering Dekkera/Brettanomyces from some wine samples despite high 4-ethylphenol concentrations. The Industry Services Laboratory has identified a number of such wines during their survey of Australian wines obtained as bottles and barrel samples. The phenomenon 'viable but non-culturable state' is a well-known problem in the food industry whereby processed foods are often spoiled by 'cryptic' spoilage microorganisms which are difficult to cultivate. We encountered a situation with this characteristic when attempting to isolate acetic acid bacteria from bottled red wine (Institute publication #718). To test for this phenomenon, Dr Peter Costello, assisted by Peter Graves, commenced a series of tests designed to resuscitate viable but non-culturable Dekkera/Brettanomyces yeast. To date, we have evidence that this is the case for the one wine studied which contains over 800 ppb 4-ethylphenol. A genetic fingerprint of the isolates will be made to negate the possibility that a variant form or a different strain/species of yeast has been recovered. These are early indications and need to be verified for other wine samples.

Microbial spoilage of bottled red wine – preliminary evidence for the role of the cork closure

In collaboration with a large wine company we previously investigated the microbiology of a series of batches of red wine which had become oxidatively spoiled post bottling (*Technical Review* issue #127). The bottles had been stored in an upright position for various periods of time within a year as opposed to storage in a horizontal position or being sealed with a ROTE closure. A low proportion of bottles, at random, in various wine batches exhibited various degrees of oxidative spoilage, ranging from loss of fruit to overt oxidation (acetaldehyde, ethyl acetate and acetic acid), which was confirmed by chemical analysis of spoiled and non-spoiled wines.

Spoiled bottles were identified by a characteristic deposit on the inner surface of the neck of upright stored bottles, giving a ring-like appearance when the bottle was tilted (Figure 8). Bacteria could be isolated from both spoiled and non-spoiled wines, and were found to belong to a closely related group of *Acetobacter pasteurianus* strains, based on molecular identification techniques developed for the project (Institute publication #718). However, oxidative metabolism of ethanol only occurred in a small proportion of bottles, apparently distributed at random within a bottling run. As similar bacteria were present in spoiled and non-spoiled bottles, we hypothesize that the upright bottle storage position created a heterogeneous environment which allowed the growth of bacteria in only those bottles sealed with cork closures that had an upper limit for the natural permeability to oxygen. Such an heterogeneous environment would not exist in horizontally stored bottles since the larger volume of wine adjacent to the cork would strongly compete with the bacteria for the oxygen as it diffuses through the cork closure. This hypothesis, which still requires careful experimental verification, has been recently published (Institute publication #718).

Although the incidence of this type of wine spoilage is unknown and likely to be relatively low, a second occurrence of a large batch of bottled Shiraz wine recently has provided us with the opportunity to test our hypothesis. The spoiled wines show similar characteristics to those of the previous study, and again a closely related group of acetic acid bacteria were found. The degree of visible spoilage varied amongst bottles and examples are shown in Figure 8. Measurement of the dissolved oxygen concentration of visibly spoiled and unspoiled bottled wines showed a difference of up to ten-fold between the two sets of wines. Sensory analysis of the wines correlated well with the higher acetaldehyde, ethyl acetate and acetic acid concentrations of severely spoiled wines. A small but significant decrease in alcohol content was also noted in heavily spoiled wines, consistent with the oxidation of ethanol. The presence of bacteria and concentrations of acetaldehyde, ethyl acetate and acetic acid correlated well with the degree of wine spoilage, as noted by the thickness of the ring-shaped deposit on the neck of the bottle.

This study further links the natural variability of cork closures to oxygen permeability to differential changes in wine composition, at least in conjunction with prolonged upright storage of bottles. Direct measurement of oxygen permeation by the cork closures used in this trial could be expected to strengthen our hypothesis. These two unconnected cases of bottled wine spoilage highlight the importance of good winery practice with respect to the preparation of wine for bottling and the minimisation of storage time of bottled red wine in a vertical upright position.

Figure 8. Visible deposit of acetic acid bacteria on the neck of bottles of spoiled red wine held in a tilted position

Lysozyme and winemaking

Lysozyme, a protein that is present in various bodily fluids and that is an important component of the eukaryotic immune system, is a natural bacteriolytic enzyme. It has several potential beneficial applications in wine production for which the Australian wine industry is applying to permit its use. For example, lysozyme can be used to inhibit certain bacterial species or delay the onset of malolactic fermentation, lowering the need for SO_2 addition in such applications. Lysozyme is only effective against Gram positive bacteria (including lactic acid bacteria) and not Gram negative bacteria (including acetic acid bacteria, such as *Acetobacter*) or yeast, such as *Saccharomyces* and *Dekkera/Brettanomyces*. Our survey of the susceptibility of wine related species of malolactic bacteria, *Lactobacillus* (three species) and *Pediococcus* (three species) and four strains of *O. oeni* to lysozyme in white and red wine did, however, indicate significant intragenic variability, with *O. oeni* strains being especially susceptible.

Research conducted in collaboration with Fordras A/S, a European manufacturer of lysozyme, has highlighted the potential use of lysozyme to control bacterial growth in both white and red wine, and also identified areas where caution is needed. The antibacterial action of lysozyme is relatively high at wine pH (~3-4) and unlike sulfur dioxide, lysozyme is also effective at higher wine pH. The use of lysozyme in winemaking can only replace the antimicrobial activity and not the antioxidant activity of sulfur dioxide. The factors that affect lysozyme activity in wine, highlighted in Table 4 and recently published (Institute publication #728), include initial bacterial cell numbers, species and strain, wine pH, phenolics content and the use of bentonite. Our research has demonstrated that there is minimal impact of lysozyme on the sensory properties of both red and white wine, an observation also noted by other research groups.

Yeast interaction with grape phenolics and effect on wine sensory properties

Staff: Dr Eveline Bartowsky, Simon Dillon, Professor Peter Høj, Dr Paul Henschke

A collaboration between the Institute and Lallemand has continued to investigate the interactions between yeast and grape phenolics during red wine fermentations. Lallemand is contributing a substantial sum of money for our investigations, for which we are most appreciative. The donation of Shiraz grapes by the Hardy Wine Company and Orlando Wyndham for these studies is also appreciated.

The background to this project, development of a micro-vinification methodology and comparison of its performance with pilot scale winery fermentation, and survey of commercial Lallemand yeast strains for impact on wine colour and phenolics content has been described in previous annual reports. The findings of a survey of 17 Lallemand *S. cerevisiae* strains for effect on wine colour density and phenolic concentration of a young Shiraz wine was summarised in the previous annual report. Analysis of the results show that the yeast strains could be divided into three statistically significant groups, that is, yeast that gave low, medium or high wine colour density. We further confirmed these results with Shiraz fruit obtained from other viticultural regions, and with frozen versus fresh fruit. These results suggested that there are intrinsic differences between some yeast strains, confirming the observations by various winemakers and Lallemand.

Table 4. Factors affecting lysozyme activity and wine stability

Wine variable	Effect on lysozyme activity or wine stability
Wine type	Effective in both red and white wines
Species/strain of lactic acid bacteria	Variation in susceptibility between genera, species and strains of bacteria
Lactic acid bacteria initial concentration	Effective against most species and strains of lactic acid bacteria up to 10^6 cfu/mL
Wine pH	Activity increases as the wine pH increases
Wine alcohol content	No influence on lysozyme activity
Phenolic compounds	Wine phenolics may precipitate with lysozyme and decrease its activity
Protein stability (haze)	May induce haze formation in white wines
Bentonite	Decrease in antimicrobial effect if used in conjunction with bentonite
Other fining agents (carbon, silica sol, oak chips and tannin)	These can bind lysozyme and reduce its activity
Sparkling wine foaming properties	In some cases may slightly increase foam stability
Other wine additives	A heavy haze may form when used in conjunction with metatartaric acid
Organoleptic properties of the wine	Minimal impact on aroma and palate has been reported

Comparison of the micro-vinification methology, based on 1 kg of fruit, with pilot scale winery fermentation using 600 kg fruit has shown that under certain conditions wines of comparable composition can be obtained, as given in the previous annual report. The trial was conducted with 2002 Cabernet Sauvignon fruit harvested from the Orlando Wyndham Padthaway vineyard in the Hickinbotham Roseworthy Wine Science Laboratory (WSL) in conjunction with the Institute's Tannin team. Nevertheless, it is necessary to confirm the magnitude of effect that yeast strains have on colour density and phenolic composition when conducted on a winery scale, and especially to determine the sensory properties of wines made with these yeast under controlled conditions.

This vintage, a pilot-scale vinification trial was again conducted in the WSL under the supervision of winemaker Stephen Clarke with assistance from Chris Day in conjunction with the Tannin team supervised by Dr Markus Herderich. Shiraz grapes from Banrock Station, Berri/Riverland, were generously provided by the Hardy Wine Company. Three yeast strains, identified from previous trials, were used to produce the wines with replicated batches of must processed in 900L capacity rotary fermentors. Alcoholic and malolactic fermentations proceeded efficiently with each of the three yeast strains. The Shiraz wines are currently being bottled for sensory analysis later this year. From initial colour and phenolic analysis, the low and high colour yeast strains produced wines that were significantly different in wine colour density and pigmented polymers (Figure 9), corroborating our earlier findings with the micro-vinification system.
The success of the pilot scale vinfication trials conducted in the WSL has depended on several important factors. First, enormous commitment and cooperation of the collaborating winery not only for providing a large quantity of fruit to the Institute's specification, but also for organising grape harvest such that 10 tonnes of grapes was divided across multiple grape bins to achieve a more uniform distribution of must composition in the winery. The Hardy Wine Company Management (specifically, Peter Dawson and Angus Kennedy) is gratefully acknowledged for generously providing the fruit as are Ben Vagnarelli and Mark Zeppel who organised the logistics of fruit harvest and delivery. The University of Adelaide's winemaker Stephen Clarke and winery assistant Chris Day are thanked for processing the must and producing bottled wine to specification in the WSL. Close cooperation of the AWRI-Lallemand project and Wine Microbiology teams, Simon Dillon, Eveline Bartowsky, Jane McCarthy and Paul Henschke, and the Institute's Tannin, NIR and Sensory teams, Markus Herderich, Leigh Francis, Mango Parker, Mariola Kwiatkowski, Daniel Cozzolino, Bob Dambergs and Kate Lattey was essential. This trial has again highlighted the need for careful planning and a large team of highly motivated individuals in order to successfully undertake a large-scale project.

Selection and improvement of wine yeasts by application of molecular biology

Staff: Miguel de Barros Lopes, Jenny Bellon, Jeff Eglinton, Lorelie Flood, Jaromir Guzinski, Anthony Heinrich, Kate Howell, Eveline Bartowsky, Dimitra Capone, Daniel Cozzolino, Gordon Elsey, Alan Pollnitz, Tracey Seibert, Mark Sefton, Paul Henschke, Sakkie Pretorius, Peter Høj

Collaborators: Dr Paul Chambers, Victoria University of Technology, Professor Graham Fleet, University of New South Wales, Dr Vladimir Jiranek, University of Adelaide, Mr Jelle Lanstein, University of Adelaide, Dr Carolyn Leach, University of Adelaide, Dr Derek van Dyk, Australian Proteome Analysis Facility

The molecular biology team is focusing on providing improved wine strains, with an emphasis on generating yeast that produce wines with improved sensory qualities. Research is continuing on three projects from previous years: the generation of a low ethanol wine yeast, the production of interspecific hybrids that impart diverse flavours to wine, and the identification of genes for wine yeast improvement. More background information on these projects can be found in previous Annual Reports of the Institute. A new project, to understand the mechanism of volatile thiol release by yeast is being performed by Kate Howell, a PhD student from The University of New South Wales who recently joined the team. Kate Howell is co-supervised by Professor Graham Fleet from The University of New South Wales and Dr Eveline Bartowsky. Productive collaborations are also continuing with Dr Vladimir Jiranek from The University of Adelaide.

Construction of a wine strain producing less ethanol

Strains overexpressing the glycerol synthesis gene (*GPD2*) accumulate more glycerol and produce less ethanol than a non-modified strain during fermentation. These modified strains, however, also increase the acetic acid concentration in wine above 1 g/L, a concentration that is generally unacceptable in wine. The deletion of a second gene (*ald6*Δ), which encodes an aldehyde dehydrogenase, provided an effective remedy for reducing acetic acid to acceptable levels. Altering the yeast's glycerol and acetic acid metabolism has been shown to have a dramatic effect on the biosynthesis of a number of other potential flavour metabolites (Institute publication #684).

The information gained above by means of a molecular genetic approach indicates that yeast are able to divert considerable amounts of carbohydrate towards glycerol production at the expense of ethanol production without necessarily having a parallel increase in volatile acidity. Lorelie Flood is, therefore, now taking a non-GM approach to generate low ethanol wine strains, which can then be used immediately by the industry. Two commercial wine yeast strains with mutations have been isolated, and these mutants show improved growth under selective conditions. It needs to be determined whether these strains are low ethanol producing strains and what other metabolic changes occur in these mutants.

Hybrid wine yeasts

Interspecific non-GMO hybrids between commercial wine yeast (*Saccharomyces cerevisiae*) and other related *Saccharomyces* species have been made by Jenny Bellon (Institute publication #685). These hybrid strains have been shown to ferment grape juice efficiently in both small-scale winemaking and commercial trials. Sensory analysis demonstrates that the hybrid-made wines were notably different to the wines made using a commercial wine yeast, with the hybrid wines being generally preferred and considered to be more complex. Using gas chromatography/mass spectrometry (GC/MS) Jeff Eglinton and Alan Pollnitz have compared the concentrations of several potential flavour metabolites in ferments made by a commercial wine yeast (AWRI 838), a *S. paradoxus* yeast or a hybrid between these two strains. Some metabolites showed a large variation in concentration between ferments of the two parental strains. For example, dihydro-2-methyl-thiophenone, a compound with a blackcurrant aroma, and 2-phenyl ethyl acetate (honey aroma) are produced at a much higher (20-fold) and a much lower (one sixth) concentration respectively in the *S. paradoxus* parent than the wine yeast. The hybrid wine showed either an intermediate concentration or a concentration comparable to one of the parents. These results demonstrate that interspecific hybrids offer an effective strategy for acquiring yeasts with novel winemaking characteristics.

The generation of hybrids between a commercial wine yeast and *S. bayanus* strain AWRI 1375 has been successful. The winemaking properties of the *S. bayanus* yeast has been well studied, and although less robust than commercial wine strains, appears to have interesting organoleptic properties. Preliminary wine fermentation results indicate that the hybrid yeast ferments as efficiently as the commercial wine yeast. The quantification of key aroma compounds are currently being analysed in these wines.

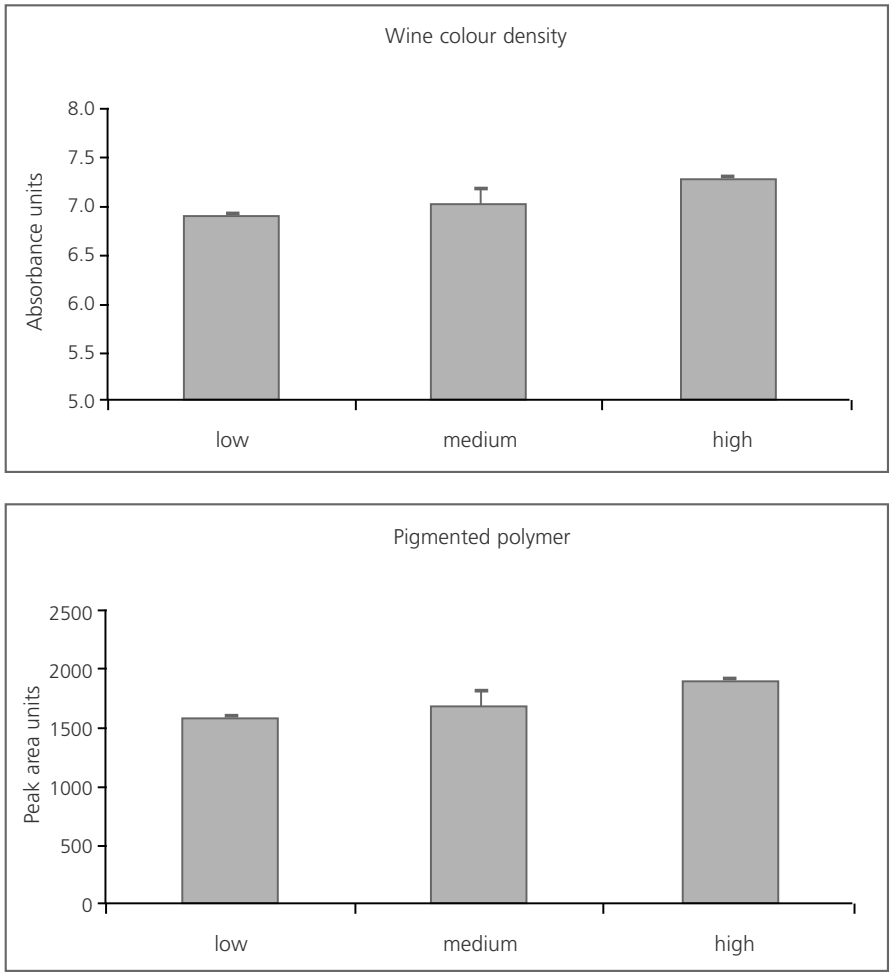


Figure 9. Comparison of wine colour density and 'pigmented polymers' of young wines made from Shiraz grape must (Berri/Riverland, 2003) by fermentation in 900L capacity rotary fermentors with *Saccharomyces cerevisiae* strains previously identified through small-scale fermentations to give wine with low, medium and high colour density

An honours student, Jaromir Guzinski, from The University of Adelaide, Department of Genetics, is characterising a hybrid isolate that has lost a chromosome from one of the parents. This hybrid appears to ferment more efficiently than the parental hybrid strain. These results are important as they may offer a means to alter the hybrid genome so that the novel aromas and flavours of the non-*S. cerevisiae* parent can be enhanced.

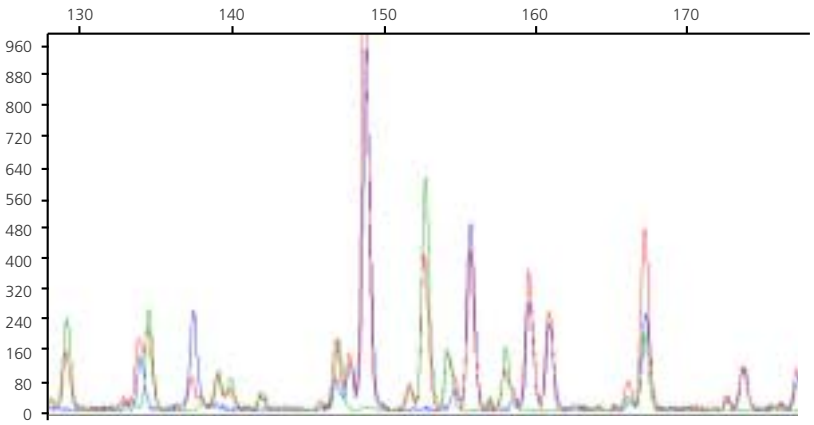


Figure 10. AFLP of hybrid wine yeast. Fingerprint of the commercial wine yeast S6U (shown in red) is a combination of the *S. cerevisiae* (shown in blue) and the *S. bayanus* (shown in green) fingerprint.

Natural interspecific hybrids also exist. Our previous studies using amplified fragment length polymorphism (AFLP) have demonstrated that the commercial wine yeast S6U is a hybrid between *S. cerevisiae* and *S. bayanus* (Figure 10). Recent AFLP studies performed by Anthony Heinrich and Jenny Bellon have identified a *S. cerevisiae X Saccharomyces kudriavzevii* hybrid wine yeast. This is the first time a commercial wine yeast possessing *S. kudriavzevii* DNA has been identified.

Identifying genes for wine yeast improvement

Saccharomyces cerevisiae is an important model organism for scientific research, and for this reason an extensive amount of knowledge on its biology is available. Although representing the same species, wine strains have important physiological differences when compared to laboratory strains. The wine habitat is a demanding environment and wine yeast need to be able to withstand a number of stresses, including the high concentrations of sugar and ethanol at the beginning and end of fermentation respectively. Furthermore, commercial wine strains have been chosen for the synthesis of metabolites that are consistent with wine aroma and flavour.

The genes and proteins that contribute to the desirable attributes of wine strains are generally unknown. Two dimensional gel electrophoresis has been used to demonstrate that the protein expression of wine and laboratory strains is markedly different. Insights into what proteins are differing in quantity between laboratory strains and wine strains might thus point to how strain performance can be further enhanced in relation to issues such as stuck fermentation. Identifying proteins of interest is technically demanding and for this research Anthony Heinrich, a PhD student from The University of Adelaide, spent six weeks at the Australian Proteomics Analysis Facility (APAF), located at Macquarie University in Sydney. Using matrix-assisted laser ionisation/desorption time-of-flight spectrometry (MALDI-TOF) a number of proteins have been identified. A collaboration with Derek van Dyk at APAF is continuing, and presently other proteins are being identified using MS/MS. Although many proteins have been identified, three proteins have been chosen for further study, a glycolytic protein, a protein involved in one carbon metabolism and a third protein that, although highly expressed in a wine yeast, is only expressed in a laboratory strain when cells respond to high concentrations of ethanol. To further explore the role of these proteins the expression of these genes are being analysed using Real Time PCR and Western analysis.

It is expected that the observed changes in protein expression may also affect the metabolites produced by the wine yeast during fermentation and by doing so, contribute to wine flavour and aroma. Preliminary results using Near Infra-Red Spectroscopy (NIR), performed with Daniel Cozzolino and the NIR team, indicate that strains lacking the differentially expressed proteins support the prediction.

We have also been fortunate to have Dr Paul Chambers from The Victoria University of Technology in Melbourne visiting the laboratory for an extended stay. Over the last few years, Paul and his colleagues have been interested in understanding how yeast acquire ethanol tolerance. Comparing the transcriptome data from Paul's laboratory to the proteome data obtained by Anthony has been invaluable.

Volatile thiol formation by yeast during fermentation

The important role of yeast in modifying the chemical, mouth-feel and flavour complexity of wine is gaining increased recognition. A class of yeast metabolites that have an important influence on wine aroma and flavour are the sulfur containing volatile thiols. These compounds have been found in a number of red and white wines, and have been shown to contribute significantly to varietal aromas of Sauvignon Blanc wines. One of the most potent thiol compounds is 4-mercapto-4-methylpentan-2-one (4MMP), which has a perception threshold of 0.1 ng/L and 3 ng/L in water and wine respectively. Depending on the concentration, this compound has an aroma of blackcurrant or cat urine/box tree. 3-Mercaptohexan-1-ol (3MH), having a passion-fruit aroma, is another important thiol that has been found at concentrations approximately 100-fold higher than its threshold concentration.

The thiols are predominantly, if not exclusively, present in grape juice as non-volatile cysteine conjugates. Predicting the concentration of the flavour precursors in grapes is not possible at present. However, in Sauvignon Blanc grapes it has been reported that the concentration of 3MH precursor is approximately 10 times higher in the grape skin than in the juice. Taking into account the respective proportions of juice and skin, this means that approximately 60% of the compound is in the skin. The 4MMP precursor is found mostly in the juice as it is present in similar concentrations in skin and juice. The conversion of the non-volatile grape precursors to volatile thiols requires enzymic action. Although the extraction of the cysteine conjugated precursors into the juice appears to be correlated to the final concentrations of the volatile thiols present in wine, only a small and varying proportion of the precursor is converted to the active aroma compound during fermentation. This conversion appears to be yeast strain dependent.

A project has been initiated to provide winemakers with the ability to regulate the amounts of the volatile thiols released during fermentation. Together with Gordon Elsey, Mark Sefton and Tracey Siebert, sensitive methods using the GC/AED (Gas Chromatography/Atomic Emission Detector) have been developed to accurately measure the concentrations of 4MMP and 3MH in model fermentations. With these methods, it has been shown that commercial strains differ greatly in their ability to release 4MMP (Figure 11), confirming observations of Dubordieu et al. in Bordeaux. The mechanisms of release of the volatile thiols from the cysteine bound conjugates are being studied. It is expected that this will provide winemakers with the knowledge to select specific commercial yeast strains and to use various winemaking practices that will produce wines with the desired concentration of these important aroma compounds.

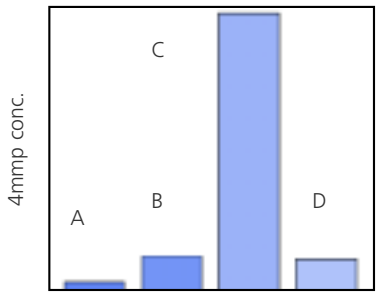


Figure 11. 4MMP release by different *S. cerevisiae* strains

Studies on unstable wine proteins involved in haze formation

Staff: Dr Elizabeth Waters, Dr Miguel de Barros Lopes, Professor Peter Høj, Ken Pocock, Shauna Brown, Yoji Hayasaka, Gayle Baldock, Richard Muhlack, Jean McIntyre

Collaborators: Dr Filomena Pettollino (CRC for Bioproducts), Dr Chris Colby, Dr Brian O'Neil (School of Chemical Engineering, The University of Adelaide), Audrey Lim (Hardy Wine Company), Dr Eileen Scott, Dr Belinda Stummer (School of Agriculture and Wine, The University of Adelaide), Natalie Fryar (Yalumba Wines)

Visual clarity is important to white wine quality. Sediments and hazes can form after bottling due to the denaturation of grape protein. Winemakers usually remove protein from wine by fining with bentonite clay and although this process is effective it has several drawbacks, including losses of wine in bentonite sediment, problems of disposal of bentonite waste, and possible effects on wine flavour. Work on improving bentonite efficiency has, therefore, been initiated this year. In addition, we have continued work on the use of haze protective mannoproteins from yeasts as alternatives to bentonite fining. Further progress has also been made on putting the 'nuisance' grape proteins to work as markers of grape variety.

Improving the efficiency of bentonite fining through chemical engineering

As the first step in improving bentonite efficiency, studies with our collaborators at the School of Chemical Engineering, have been undertaken on how proteins absorb onto bentonite. Data from initial studies with a model protein, BSA, as part of the method optimization experiments, has suggested that absorption is pseudo irreversible. This supports work by others demonstrating that removal of absorbed protein from bentonite lees is difficult and that bentonite regeneration is not a simple process.

We plan to study the absorption onto bentonite of the two main unstable wine proteins, grape thaumatin-like proteins and chitinases. These proteins are not commercially available so purification of these proteins from a Muscat Gordo juice concentrate has commenced.

Use of haze protective mannoproteins as an alternative to bentonite fining

The putative haze protective mannoprotein genes from yeast (*HPF1, HPF1'* and *HPF2*) have been cloned and all three have been successfully deleted individually and in combination in haploid and diploid laboratory yeast strains. Analysis of the haze protective activity of the material from over expression and deletion strains has supported our identification of these genes as coding for haze protective factors. By using a hexahistidine labelled form of Hpf2p (6xhis Hpf2p) we have also obtained evidence that we have successfully identified a gene coding for a haze protective factor.

Phenotypic analyses have suggested that the haze protective mannoproteins may have some impact on the ability of yeast to tolerate cold and ethanol. This is an unexpected bonus from the HPF project and illustrates the serendipitous nature of research. Further investigation of the deletion strains has suggested that Hpf1p and Hpf1'p are also involved in yeast mating.

A heat unstable Sauvignon Blanc wine containing a hexahistidine labelled form of Hpf2p (6xhis Hpf2p, 200 mg/L), invertase (200 mg/L) or no addition ('control') has been prepared and stored at 25°C. After three and six months' storage, both 6xhis Hpf2p and invertase appeared to be as active as they were when the trial was set up, indicating that the short term stability of these haze protective mannoproteins in wine is good, even at slightly elevated temperatures.

Haze protective mannoproteins during secondary fermentation

Industry practitioners have observed that protein unstable base wines become stable immediately following secondary fermentation. A trial was initiated in December 2001 in collaboration with Natalie Fryar, Yalumba Wines, to explore this phenomenon. One base wine was treated with either none or two different levels of bentonite. These lots were put through secondary fermentation and aged on lees under commercial conditions.

The base wines treated with both rates of bentonite were heat stable, and these bottled lots remained so during secondary fermentation and ageing. The unfined lot was not stable during secondary fermentation, and this lot remained heat unstable, on the basis of an 80ºC six hour heat test, throughout the experiment. However, the heat stability of this wine improved following tirage even though the amount of unstable wine proteins was unchanged. The levels of haze protective mannoproteins in this wine appeared to be greater at four weeks post tirage than at the beginning of the trial.

These results suggest that improvement in heat stability of wines following secondary fermentation may be due to additional release of haze protective factors by yeast. Whether this wine, with improved heat stability would be considered stable enough for commercial release is a decision that needs to be made after trials by individual companies and would depend on their specifications.

The mechanism of haze protective activity

Analysis of the size of particles in unheated and heated wine samples containing 6xhis Hpf2p showed that there was a decrease in particle size with increasing 6xhis Hpf2p concentration both before and after heating when compared with the control. These trends are similar to those observed previously with less pure samples of haze protective factors (Institute publications #439, 639) and indicate that Hpf2p, like other haze protective mannoproteins, reduces visible haziness by effecting protein aggregation and final particle size. The mechanism by which this occurs is not understood but the team at CRC for Bioproducts are continuing to study the mechanism of action using physical (laser light scattering, atomic force microscopy, GPC MALLS, etc), chemical and biological (protein and carbohydrate chemistries, enzymic digestion, etc.) techniques. The aims are to find out how it works and which part of the Hpf2p molecule is responsible for haze protection. This could allow non-GMO production of haze protective material.

Use of mass spectrometry to differentiate varieties

The ability of the method to differentiate the variety of grapes from analysing wine was further confirmed this season. Five white and five red wines were vinified from grapes of different known varietal origin. By HPLC, apart from Merlot, none of the red wines we have analysed contained detectable proteins, although low levels of proteins could be detected in the juice from red grapes. Despite this very low level of protein, it was possible for the mass spectrometry operator to correctly identify the grape variety for the Merlot wine and for all of the non-fined white wines by mass spectrometry without prior knowledge of the varietal identification.

Bentonite fining presents a particular challenge to this method. White wines stabilised with bentonite contain low but detectable levels of the thaumatin-like proteins but do not contain detectable levels of chitinases, the most varietally variable group of wine proteins. The differences in the Mr of thaumatin-like proteins alone are probably not sufficient for varietal differentiation but could be used to exclude the presence of certain varieties in a wine (see also Institute publications #663 and 734).

Work performed last vintage by a visiting student from the University of Barcelona, Teresa Girbau, and followed up this vintage in collaboration with Eileen Scott and Belinda Stummer at the School of Agriculture and Wine, The University of Adelaide through CRCV Project 1.5.2, has shown that powdery mildew infection increases the levels of one of the thaumatin-like proteins in Chardonnay juice and wine. At higher levels of infection (>30% of bunches infected), this has a significant impact on the level of haziness in the wine following a heat test. Despite the quantitative changes in protein levels in juice and wine due to powdery mildew infection of grapes, the characteristic qualitative protein profile was maintained. We, therefore, currently see no reason for powdery mildew infection of grapes to effect the ability of the electrospray mass spectrometric method to varietally differentiate grapes.

Studies on random oxidation of bottled wines

Staff: Dr Elizabeth Waters, Dr George Skouroumounis, Mariola Kwiatkowski, Dr Leigh Francis, Kate Lattey, Dr Mark Sefton

Collaborator: Andrew Kleinig (Southcorp Wines)

Oxidative degradation of white wines is a problem for winemakers worldwide and results in loss of characteristic aromas of young wines, namely their floral and fruity notes, and the development of brown colour. It differs from ageing, which is largely the result of various chemical reactions, by being primarily caused by reactions promoted by oxygen. As an example of this, it has been found in a recent study that oxidised Riesling lost its floral and citrus notes and became aldehydic and brown in colour, whereas aged Riesling also lost the floral and citrus notes but acquired typical bottle age characters such as honey, toasty, cooked citrus/lime and developed a golden colour.


Mariola Kwiatkowski, Patrik Jones & Elizabeth Waters

After fermentation, the main cause of oxidation is contact of the wine with oxygen. This can occur during normal transfer operations and barrel storage. Adequate SO_2 levels should prevent excess oxidation during these operations (Robinson and Godden, *Technical Review* 145). One additional and more recently identified route for browning is that of oxygen permeation into bottled wines through the closure and/or closure/glass interface (Institute publications #534 and 580). Data from the Institute's Closure Trial (Institute publication #666) also suggest that closures and/or the closure/glass interface vary in the amount of oxygen permeation they allow. Due to this variability, the resulting oxidation of wine in bottles can be seemingly random within a batch.

The aims of this project are to identify bottling and storage procedures to minimise oxygen contact with wine and its effects if oxygen contact occurs, and the parameters of the closure and closure/glass interface that influence its oxygen permeability.

Oxygen during bottling and storage of white wines

A Riesling and a wooded Chardonnay wine were bottled in August 1999 for an experiment undertaken with assistance from Southcorp Wines to evaluate the effect of ascorbic acid, bottle position and wine type on the extent of oxidation. Bottles were sealed with either a synthetic closure, a ROTE (roll-on tamper evident) or one of two Reference 2 natural corks from different suppliers. Measurements of A 420 nm on at least 30 replicate bottles for each treatment have been taken throughout their storage period using a modified spectrophotometer (Institute publication #731).

A descriptive analysis study of wine colour after two and a half years' storage confirmed that A 420 nm measurements were not valid as a measure of brown colour when comparing wines with and without ascorbic acid. However, when comparing the same wine, a good correlation was observed between wine colour and A 420 nm values.

The absence of ascorbic acid at bottling and upright storage for two and a half years under ideal conditions, in general, gave wines with the highest ratings for brown and orange colour. The extent of the effect of storage position on wine colour depended on the closure type, with the synthetic closure much less affected by position than the natural bark closures.

A second large sensory investigation on the aroma characteristics of these wines was undertaken in August 2002 when the wines were close to three years old. This study showed that the visually browner wines have more oxidised aroma. The wines without ascorbic acid were in general rated higher in oxidised character than the wines with ascorbic acid. The ratings for oxidised character were also negatively correlated with fresh fruit characters in both Chardonnay and Riesling, a result also seen with the Semillon wine in the Institute's Closure trial.

For the Chardonnay wines, the free and total SO_2 levels after three years' storage were marginally lower in wines to which ascorbic acid was not added at bottling. Closure type also affected the free and total SO_2 and ascorbate levels, with the synthetic closure having the lowest levels of these compounds and the ROTE (roll-on tamper-evident) closures having the highest. These results were in line with the sensory data. There was no effect of storage position on ascorbic acid levels in wines. The level of free SO_2 was lower in all wines stored upright but the effect of storage position on total SO_2 levels depended on ascorbic acid addition at bottling – there was no effect if ascorbic acid had been added at bottling. Similar results were observed for the Riesling wines.

In summary, the chemical and sensory data from this trial clearly indicate that for all four closures examined, these wines were less oxidised after storage under ideal conditions for two and a half to three years when ascorbic acid was added at bottling. This information was presented at the 2002 Roadshows (see Appendix 1) and will be written up for publication in the *Australian Journal of Grape and Wine Research*.

The effect of ascorbic acid addition on oxidation of wine continues to be a matter for debate in the literature. Previous laboratory scale work from the Institute (Institute publication #595) and others indicated an enhanced depletion of sulfur dioxide in wine and model solutions to which ascorbic acid was added. Formal sensory assessment, however, has rarely been conducted in these previous experiments and the conditions of the experiments were not always closely aligned to commercial practice. The current study, through collaboration with a winery, has allowed us to examine the effect of ascorbic acid addition to a Riesling and a Chardonnay wine bottled on a large scale under commercial conditions. Under these conditions, aroma, colour and compositional data all indicate that the wines were less oxidised after storage under ideal conditions for two and a half to three years when ascorbic acid was added at bottling. When analysed after six months' storage, there was little difference between the wines (Institute Annual Report 2000).

Oxygen during bottling and storage of red wines

A preliminary experiment was initiated to gather information about whether a red wine under screw cap develops more reduced aroma, altered colour and sensory properties than under natural cork or synthetic closures and to evaluate whether headspace volume under the screw cap effects these differences.

A commercial Cabernet Sauvignon wine provided by Southcorp Wines, and with Dr Andrew Kleinig's assistance, was bottled on 16 December 2002 with a screw cap (Auscap) with headspaces of 4, 16 and 64 mL air. As a comparison, the same wine was bottled and sealed with natural cork (Reference 2) and a synthetic closure using vacuum insertion and an ullage of 6 mL and 5.4 mL, respectively. The composition of the wine at bottling was typical for wines of this style. The wines are being stored upright at the Hickinbotham Roseworthy Wine Science Laboratory (WSL).

A check on the SO_2 levels occurred six weeks after bottling in late January 2003. The SO_2 levels in wines sealed with the natural bark closure, the synthetic closure and with the screw cap with a 4 mL headspace were very similar. As the headspace volume increased under the screw cap, the SO_2 levels reduced. A further check on SO_2 levels was undertaken at five and a half months after bottling. At this very early stage, the highest SO_2 levels were seen in the wine sealed with the screw cap with a 4 mL headspace, intermediate and similar SO_2 levels were seen in the wines sealed with natural cork, synthetic closures and the screw cap with a 16 mL headspace and lowest levels in the wine sealed with the screw cap with a 64 mL headspace.

A tasting panel at the Institute informally assessed the wines after six weeks. At this early stage, reduced aromas were not obvious in any wines, including the wines with 4 mL headspace under screw cap, and it was difficult to see differences among the treatments. Five and a half months after bottling in mid June 2003, informal assessment again suggested that differences among the treatments may not be obvious and reduced aromas were not clearly apparent to all tasters. Formal duo-trio difference testing for aroma was undertaken to confirm these observations and the wines sealed with natural bark closure were compared with the other treatments. None of the wines sealed under the screw cap or the wine sealed with the synthetic closure were judged by the panel to be different from the wine sealed under the natural bark closure at this early stage of development. These wines will be assessed again at a later date.

The inherent permeability of corks to oxygen

As reported previously, a spectrophotometer has been modified to measure wine colour, the result of oxygen permeation, whilst the wine is in the bottle. A paper describing this method has been published in the *Australian Journal of Grape and Wine Research* (Institute publication #731).

Chemical assays to quantify the relatively low levels of oxygen permeating through closures in wine bottles are not readily available or simple to develop. The basis for our current and longstanding effort is to develop an assay using a compound that acts as a water-soluble trap of singlet oxygen. Good progress this year has been made on developing the assay and optimising the experimental conditions with the help of Christophe Guirado, a visiting student from ENSBANA, Dijon, France.

Gayle Baldock & Yoji Hayasaka

A preliminary experiment using the method to monitor oxygen permeability into wine bottles sealed with a synthetic cork was set up in February 2003. Data collected to date indicates that this assay may indeed allow us to estimate oxygen permeation rates within four to eight weeks. The advantage of this method over other commercially available systems is that the closure is in contact with a wine-like solution rather than being in a gaseous environment. Thus the performance of closures in wine bottles can be assessed in a range of storage environments that relate directly to commercial use.

As previously reported, araldite, a material well known for its oxygen impermeability, was applied to the tops of bottles over the glass and the closure to either completely cover the closures of two replicate bottles, or to partially cover the closure so that a 'window' remained in the centre of the closure for four replicate bottles. The aim of this experiment was to investigate the route of oxygen permeation into wine bottles (through the closure or the closure/glass interface?). After nine months' storage, the browning of wines in bottles with closures with the 'windows' was similar to that in the bottles with the fully covered closures for the natural closures and higher than that shown by wines in bottles with the fully covered closures for the synthetic closures.

A more extensive experiment using araldite has been subsequently set up to build further on these earlier observations. Araldite has been used to either fully cover, cover 80%, 50% or 20% of the surface of the closure either as a circle in the centre of the closure, or as a ring around the edges, or to cover 50% of the closure as a semicircle. One wine sealed with one of two closures (a natural cork or a synthetic closure) has been used for this experiment and each of the nine araldite treatments including the control (no araldite coverage) has at least seven replicate bottles. The wines were bottled at Southcorp with the help of Andrew Kleinig and then the araldite treatments applied at the Institute. The wines are stored upright at 25°C.

Wine colour measurements (A 420 nm, *in situ*) were made every week in the first month then fortnightly until the fifth month and are now being made once every six weeks.

So far, after nine months' storage, no clear differences in A 420 measurements among all the treatments have become evident, however, as expected, the trend is for wine colour to be related to the area of the closure covered. It is likely that SO_2 levels need to be depleted sufficiently before colour differences become large.

Waite Campus Mass Spectrometry Facility

Staff: Yoji Hayasaka and Gayle Baldock

The four important roles of the Waite Campus Mass Spectrometry Facility are to act; (i) as a leader in the application of mass spectrometry to grape and wine research; (ii) as an investigator to solve the problems facing the wine industry and individual wine makers, using mass spectrometric techniques; (iii) as a collaborator with The University of Adelaide in the research and teaching activities involving mass spectrometry and (iv) as a provider of the versatile and advanced mass spectrometric techniques and expertise to the scientific community including public institutions.

Mass spectrometry facility usage trends

The application of the electrospray technique has continued to increase in the reporting period. This technique is an indispensable tool for the Institute's protein and tannin projects and has become a useful tool for problem solving. In response to the increase in demand for this application, the MS facility needs to have additional electrospray capability.

The additional electrospray capability is necessary to enhance the progress of the current projects and to prepare for future contributions to other projects and arising problem solving issues. Our future ability to collaborate with Provisor, a new technical service provider to grape and wine research,

will give us access to new equipment including electrospray mass spectrometers. It represents an opportunity for us to overcome the current and growing problem of limited mass spectrometric resources.

The Facility's TSQ GC-MS/MS and API LC-MS/MS are used for various purposes and appropriate financial arrangements for all users are in place to recover the running cost of the Facility. The usage of the TSQ GC-MS/MS and API LC-MS/MS for the year is detailed in Table 5.

Table 5. Usage of the TSQ GC-MS/MS and API LC-MS/MS for 1 July 2002 to 30 June 2003

	TSQ GC-MS/MS	API LC-MS/MS
The Australian Wine Research Institute	91.0%	77.3%
The University of Adelaide	9.0%	20.7%
CSIRO	0.0%	1.4%
Flinders University	0.0%	0.6%

Collaboration with the Industry and Analytical Services

During the reporting period, the Facility conducted mass spectrometric analyses on 100 non-research samples, in collaboration with staff of the Industry and Analytical Services. The analyses conducted during the reporting period are detailed in Table 6.

Table 6. 'Problem' investigations conducted in the period from July 2002 to June 2003

Type of investigation	Number of samples analysed
Brine contamination (Alcohol based)	35
Protein analysis for varietal identification	30
Brine contamination (Glycol based)	9
Unknown taint	8
Closure problem	6
Diesel analysis	4
Paint/varnish analysis	3
Hydraulic oil analysis	3
Kerosene analysis	1
Sorbic acid analysis	1
Total	100

Juice protein analysis for a varietal identification

It is highlighted that this new technology delivered from our research has been adopted for use by Industry Services in consultation with winemakers who submitted 30 samples to the Institute for protein analysis during this financial year. This new technology is used for varietal differentiation of juice based on the composition of pathogenesis related-proteins (PR-proteins) (Institute publications #663 and 734). This study described that PR-proteins in untreated juice were detected using electrospray mass spectrometry combined with a protein trap cartridge (Trap-MS method) and the resulting protein composition in the juice was consistent among samples representing the same variety but differed among varieties. The method was validated using juices from berries from 20 different varieties (*Vitis vinifera* cv.) harvested in at least two different seasons and from different vineyards.

The analytical results of the submitted samples demonstrated that the technology could be used as an additional tool for winemakers to ensure the varietal origin of juice. The availability of this technology is expected to place Australian winemakers in a stronger position than their overseas competitors to back up claims of integrity from vineyard to bottle.

Brine contamination

Of all 'problem investigation' analyses conducted during the year, the juice and wine samples suspected to be contaminated with brine accounted for 44% of the total analyses conducted by the Facility. In response to an increasing demand on the analysis of brine, the Facility developed two reliable methods for the detection of alcohol-based and glycol-based brine, respectively.

(1) Alcohol-based brine
The red pigment, rhodamine is one of the components in alcohol-based brine. It is present at a concentration of 10 mg/L but this concentration may vary among manufacturers. Contamination with alcohol-based brine was evaluated by the 'presence' or 'absence' of rhodamine in juice or wine and then the degree of contamination was estimated by its concentration. Rhodamine was detected using electrospray LC-MS in selected ion reaction mode after the extraction and enrichment of rhodamine using a C18 cartridge. This method is capable of detecting 0.001% brine in juice or wine, which is equivalent to 10 mL of brine in a tonne of juice or wine.

(2) Glycol-based brine
A GC-MS method was developed mainly for the detection and quantification of 1,2-propylene glycol in red and white wine. Propylene glycol was reliably quantifiable in a range of 10 to 250 mg/L. The quantification limit of propylene glycol using this method is 10 mg/L. Therefore the method is capable of detecting approximately 0.001% glycol in wine (10 mL of glycol in a tonne of wine).

Collaboration with research groups

The contribution of the MS facility to the Institute's research projects is detailed in the reports under *Wine grape tannin and colour specification* and *Studies on unstable wine proteins involved in haze formation*.

Briefly, the following outcomes are highlighted:

In collaboration with Dr James Kennedy (Oregon State University, USA), the direct condensation product (polymer) of an anthocyanin and proanthocyanidin was characterised using chromatographic and mass spectrometric techniques. The degree of polymerization was found to be as high as an octamer composed of an anthocyanin combined with a proanthocyanidin heptamer. This is the first mass spectrometric evidence confirming the existence of the direct condensation products of an anthocyanin with proanthocyanidins since Somers proposed the formation of 'pigmented polymer' in aged wine in 1971.

In collaboration with Dr Stéphane Vidal and Dr George Skouroumounis, the direct condensation products of anthocyanins were characterised using MLCCC and mass spectrometric techniques. The presence of the direct condensation products of anthocyanins in either grape skins or wine has not been reported. This is the first mass spectrometric evidence to propose the existence of anthocyanin oligomers in grape skins and wine.

The switchability of the mass spectrometric technique for varietal differentiation based on PR-proteins of both white and red grape juices has been further confirmed. The extent to which the discriminating power can be extended to white wine is limited if bentonite fining has occurred.

Kate Lattey & Leigh Francis

Contribution to the establishment of Provisor

Yoji Hayasaka and Dr Alan Pollnitz have participated in the establishment of Provisor as members of the Provisor Building Committee.

The activity of the Provisor Building Committee includes:

> To discuss matters in relation to a new building for Provisor.

> To specify and assess the existing instruments of the shareholders which can be relocated to a new building.

> To recommend the space and service (gas, water, electricity etc) requirements for labs and offices.

> To identify and procure the instruments which are required for the Provisor service activities.

> To investigate the merits of computer network integration between Provisor, AWRI and CSIRO.

Powdery mildew study

Staff: Dr Leigh Francis and Kate Lattey

Collaborators: Dr Belinda Stummer and Dr Eileen Scott, The University of Adelaide

Following from previous work, recently published in the *Australian Journal of Grape and Wine Research* (2003, 9, 28-39, Institute Publication #714), a sensory study with Belinda Stummer and Eileen Scott of The University of Adelaide was carried out with wine from the 2002 vintage. The study is part of CRCV Project 1.5.2. Chardonnay wine was made from fruit which had been sorted into four different levels of powdery mildew infection as had been done previously but, in this case, a period of skin contact was imposed to mimic transport of machine harvested fruit in a commercial situation. In addition, great care was taken to ensure that the gross composition of the wines was comparable, as the set the previous year had some variation in TSS at harvest. The composition of each sample for measurements such as alcohol was found to be very similar although there were some differences in pH and TA.

The sensory data confirmed that the wines made from powdery mildew-affected fruit, even at the lowest level of infection, were perceived to be significantly higher in a viscosity/oily attribute compared to the control, uninfected treatment, and this attribute was correlated with phenolic measures in the wines. This observation was as found in the previous study. In addition, the 'infected' treatments were rated as higher in fungal and earthy aroma compared to the control, with the wine made from the most severely affected grapes being rated the most pronounced in these attributes, with concomitantly lowered fruit attributes.

Industry Services Teams' report

Technical problem solving and consulting

Staff: Peter Godden, Adrian Coulter, Mark Gishen, Ella Robinson, Peter Høj, Geoff Cowey, Yoji Hayasaka, Trudy Wallis, Greg Ruediger and Peter Valente

The Industry Services team provides technical development and support services to the Australian wine industry, primarily in the form of an advisory service that disseminates a wide range of technical information, and a problem solving and analysis service, which collectively represent a significant proportion of the team's workload. The team continues to manage a large research trial that is examining the technical performance of various types of wine closure, and a project that has developed a web-based technical reference manual for the Australian wine industry, with associated workshops that are presented in conjunction with Institute Roadshow seminars. In addition, the team coordinates an Institute-wide project that is investigating the relationships between the potential spoilage yeast *Dekkera/Brettanomyces* and wine in Australia.

The Industry Services Laboratory analysed in excess of 2000 samples during the year, using a wide range of routine or novel analytical techniques. An increased reliance on advanced analytical methods provided by the Waite Campus Mass Spectrometry Facility, the Institute's commercial Analytical Service and our sensory panels led by Kate Lattey and Leigh Francis is acknowledged. The majority of samples analysed are wine; the analysis of which is supplemented by detailed sensory evaluation by a panel of experienced tasters. The remaining samples predominantly consist of wine additives, closures, or compounds that are suspected to have caused taints and or deposits in wine. The capability of the Industry Services team was enhanced during the year by the recruitment of Geoff Cowey (formerly employed in a large wine company as Chemist/Microbiologist) in the position of Chemist – Industry Services, and by the team gaining access to a new GWRDC funded GC-MS instrument.

The primary aim of the investigative and advisory service is to offer preventative and remedial advice based on the cumulative problem solving experience of the staff, and the practical winemaking experience of the team Manager, Oenologist and other staff members, rather than providing simple diagnoses of the causes of various problems. It is clear that quality loss during wine processing and packaging represents a major cost to the Australian wine industry, and consequently all the activities of the Industry Services team, in terms of problem solving, extension and information transfer, and research, can increasingly be seen to address this issue in a targeted manner, such as exemplified by the closure trial and more recently our 'Brett' project. To this end, staff members increasingly regard their role as educational, seeking to disseminate information in a variety of ways in order to foster industry wide understanding of the causes of many common winemaking problems, in order to prevent their frequent recurrence. The Industry Services team also provides technical support to the Institute's Analytical Service, particularly in the maintenance and auditing of the quality management system, and the interpretation of analytical results. The Analytical Service also supplies chemical analysis on problem solving and research samples to the Industry Services team, on a contractual basis.

The Institute's investigative and advisory services are provided according to strict Terms and Conditions, and client confidentiality is an important aspect of the provision of the services. This facilitates a frank exchange of information between the Institute and its clients, which in turn allows the maximisation of the knowledge gained from the provision of these services.

A proportion of the investigations conducted by the team relate to disputes arising between levy-payers or between levy-payers and suppliers of either materials or contract services. Consequently, and somewhat reluctantly, Industry Services staff members, with the involvement of the Director and Company Secretary, often find themselves in a mediation role in these disputes, and spend a considerable amount of time providing technical information to legal professionals representing grapegrowers and wine companies. It is, therefore, clear that the support offered to the Australian wine industry by the provision of these services is increasing in scope, and is of great benefit to clients, as the majority of disputes are settled before formal court proceedings are instigated.

A summary of the number and type of investigations conducted by the Industry Services team over the past three financial years is presented in Table 7. The number of investigations conducted decreased by 8% from the previous year. However, the total number of samples analysed increased by 89%. Twenty per cent of this increase is accounted for by samples analysed as part of the investigations into the effects of bushfire smoke on grapes and wines, which are described below. Notwithstanding the bushfire smoke investigations, the previously reported trend of a greater number of samples being analysed for each investigation has continued. This trend has been driven by a number of factors, including an apparent increase in the use of Industry Services reports to support insurance claims, and in legal proceedings. More recently, it has become a National Association of Testing Authorities (NATA) requirement of the Analytical Service laboratory that measurements of the uncertainty of the results of analyses be reported with each result. It is considered good practice for this policy to also be adopted by the Industry Services laboratory, and in its reporting, in order to strengthen the statistical certainty of conclusions that may be drawn in these reports. Consequently, due to the inherent degree of uncertainty of many of the analytical methods used, it is clear that a large number of samples need to be assessed in order to obtain the desired level of statistical significance.

Approximately 40% of investigations conducted during the current year related to the combined problems of hazes and deposits and microbiological instabilities. Whilst the number of wines exhibiting these types of problems as a proportion of all wines made in Australia appears to have fallen in recent years, the overall number of wines affected should remain a concern for the Australian wine industry as a whole. Issues related to such instability problems continue to be addressed by GWRDC-funded Project 99-1 *Targeted training of wine industry personnel: Compilation of a*

Table 7. Summary of the number and type of problem solving investigations conducted, and numbers of samples analysed by Industry Services during the past three years

	Investigations conducted and samples analysed		
	2000/01	2001/02	2002/03
Identification of hazes and deposits	135	85	112
Microbiological investigations	50	89	95
Sensory assessments	51	98	89
Taint problems	66	43	72
Other investigative analyses	128	209	113
Closure-related investigations	24	22	20
Total number of investigations	454	546	501
Total number of samples analysed	1048	1233	2231

technical reference manual and development of associated workshops, which is discussed in more detail later in this report. Additionally, the proportion of the problems investigated in which the apparent primary cause is an insufficient concentration of the preservative sulfur dioxide (SO_2), particularly at bottling, has increased. Consequently, Industry Services staff produced an article providing practical information on optimising the effectiveness of SO_2 additions made to wine, for publication in *Technical Review* issue 145 (August 2003).

The number of sensory assessments conducted during 2002/2003 has remained close to the record high level recorded in the previous year, and the continued demand for this type of work can be partly explained by the Institute's ever increasing capabilities in this area.

The types of investigations recorded in Table 7 as 'other investigative analyses' are extremely varied, and some particularly interesting and unusual cases have been investigated this year.

> Following a publication in a refereed journal (Institute publication #663) and a synopsis in our *Technical Review* regarding the feasibility of authenticating varietal descriptors for grape juice, the Institute has been engaged by a number of winemakers keen to implement further measures to strengthen label integrity measures in the industry. This can be particularly useful if, for one reason or the other, a winemaker fears a rare mix-up has occurred during the winemaking process. The Institute received a number of samples that were analysed by the Institute's Mass Spectrometry Manager, Yoji Hayasaka, and the Director, Professor Peter Høj, according to the method of Hayasaka et. al. 2001 (Institute publication #663). In one case, based on sensory assessment of juice, a winery was questioning whether some Sauvignon Blanc juice that it had purchased was in fact 'low ripeness Semillon', and in another, whether juice that had been returned to a winery following contract juice-lees filtration was in fact the filtrate from the lees that had been despatched. In both cases, the conclusion drawn from the protein analyses was that the juices were 100% of the variety that they were purported to be.

> A brown granular deposit isolated from a bottle of brandy was investigated. Microscopic examination of the material revealed light brown crystals with some amorphous material. No significant or characteristic absorbances were observed in the infra-red spectrum of the isolate, however, a flame test on the material produced an orange-red flame, suggesting the presence of calcium. The presence of metal in the deposit was also suggested by the fact that it was found to be soluble in 10% hydrochloric acid. Analysis of the material using atomic absorption spectrometry indicated that it contained 28% calcium. A portion of the material was dissolved in a mixture of methanol and hydrochloric acid and heated at 80°C for 30 minutes, which produced a bright red colour indicative of the presence of condensed tannins. The isolated material was, therefore, concluded to be calcium tannate, the first documented case of this type of instability investigated at the Institute for at least 14 years. Calcium is one of the most important elements causing instability in brandy and has been known to precipitate as the amorphous tannate when the concentration of calcium is greater than 5 mg/L (Institute publication #58). The main sources of calcium in brandy are water used for dilution, and leaching from insufficiently washed filter pads (Institute publication #58).

> As reported in previous Annual Reports, wines that are found to be accidentally contaminated with substances that are not permitted additives to wine is of concern, and the most common causes of contamination continue to be refrigerant brine and hydraulic oil. Readers should be aware that the Institute's contact with this type of problem is due to wineries referring wine to the Institute as a precaution if, for one reason or other, it is suspected an unintended introduction of substances has occurred accidentally. Such precaution is an integral step in the industry's duty of care to consumers. Issues related to the minimisation of the risks of this type of contamination continue to be addressed in the workshops presented during Institute Roadshows, and it is apparent that in many of the cases investigated, the contamination could have been avoided if the advice presented in these workshops had been heeded. A higher than usual number of samples was investigated for possible refrigerant brine contamination during the current year, due to brine leaks apparently having occurred in relatively large wineries, and the investigation of these samples has been greatly aided by the development of a GC-MS analysis for the compound rhodamine, which is used as a dye in several commercially available refrigerant brines. The development of this and several other GC-MS and LC-MS methods by the Institute's Mass Spectrometry Facility Manager, Yoji Hayasaka, and technical officer, Gayle Baldock, has greatly aided the Industry Services team during the reporting period. Their work is appreciatively acknowledged.

> One wine sample analysed was found to contain 1860 ng/L of TCA, the highest concentration ever recorded by the Institute's Analytical Service. The sensory recognition threshold for this compound for the experienced tasters who assessed a white Semillon wine as part of the Institute's closure trial was established to be 1 ng/L.

> An investigation was conducted into a red wine in which the concentration of volatile acids (VA) had reportedly increased dramatically in a short time period. The wine had been subjected to micro-oxygenation for 14 days while undergoing malolactic fermentation (MLF). After the wine had completed MLF, sulfur dioxide was added and the wine was then subjected to micro-oxygenation for a further eight weeks. At the end of this period the concentration of VA in the wine was 0.52 g/L. However, approximately three weeks later the concentration of VA had risen to 1.39 g/L. In the apparent absence of other markers of chemical oxidation of the wine such as the development of excessive brown colour or an elevated concentration of acetaldehyde, the chemical oxidation of ethanol to acetic acid was discounted as a cause of the dramatic increase in VA in this wine.

Microbiological analysis of the wine indicated it contained viable lactic acid bacteria (LAB), suspected to be *Lactobacillus* sp. This species of LAB is capable of causing spoilage in wine, however, it would be considered unusual for *Lactobacillus* sp. bacteria to cause an increase of approximately 0.85 g/L in the concentration of VA in a wine over a two week period, as was reported to have occurred. The wine also contained a deposit that was found to consist of non-viable yeast cells, suspected to be of *Saccharomyces* sp. and *Brettanomyces* sp., as well as rod-shaped bacteria which may have been either lactic acid bacteria, possibly *Lactobacillus* sp., or acetic acid bacteria, possibly *Acetobacter* sp. Inspection of the results of chemical analysis of the wine suggested that the combination

Geoff Cowey, Adrian Coulter & Ella Robinson

of moderate SO_2 concentration (the winemaker had made an SO_2 addition when the increase in VA was discovered, and prior to sample being submitted to the Institute), and low pH, may have been responsible for the lack of viability of the microorganisms found in the deposit.

Brettanomyces yeast are capable of causing spoilage in wine and may have been responsible for the observed increase in the concentration of VA if the yeast had been viable at the time that the increase occurred. If viable *Brettanomyces* yeast were present in the wine before MLF, then it is possible that the population of the yeast may have increased during MLF, aided by the presence of oxygen due to micro-oxygenation. Subsequently, following further micro-oxygenation, the population of the yeast may have reached a level where they were able to produce the observed increase in the concentration of VA in a short period shaped bacteria observed in the deposit were acetic acid bacteria, and that these bacteria were responsible for the increase in the concentration of VA, if it is assumed that they were viable following MLF. As with the former scenario, micro-oxygenation of the wine may have facilitated an increase in the population of acetic acid bacteria if they were present and viable during the process.

It is difficult to determine which microorganism or organisms were responsible for the increase in VA in this wine, although it is possible that the process of micro-oxygenation may have contributed to the problem. **It is the standard advice of Industry Services staff that wines undergoing micro-oxygenation should be sensorially assessed regularly, preferably on a daily basis. The results of this investigation suggest that this advice should be extended to include the weeks immediately following the cessation of micro-oxygenation.** We are not concluding that the application of microoxygenation in a correct fashion leads to dramatic increases in VA, however, we believe it important that practitioners are informed of this incident.

> During the year the Institute became aware of a number of problems resulting from the mis-use of roll-on tamper-evident (ROTE) *screwcap* closures. The problems ranged from the use of closure and bottle combinations that were incompatible, a fact that was readily identifiable by inspecting the specifications of the closures and bottles, to three instances where the force used to apply the closures appeared to have been too high. In one case, this resulted in the tamper-evident perforations on the closures being broken on some bottles, or damage to the internal lining of the closures in the two other cases. In one of these cases a dozen bottles of a Pinot Noir wine were submitted for investigation of an apparent sporadic oxidation problem. One of the bottles was opened, and was initially subjected to microbiological analysis because the sample appeared to contain variable microorganisms in the form of a deposit. Following microbiological analysis of this first sample, an informal sensory assessment of the wine was conducted and it was considered obvious that the wine contained a high concentration of volatile acids (high VA). This sample contained a 'tide line' in the form of a ring of dark coloured material adhering to the inside surface of the neck of the bottle, which was found to consist of rod-shaped bacteria, suspected to be *Acetobacter* sp., coccoid bacteria, suspected to be *Oenococcus* sp., and yeast cells, suspected to be *Saccharomyces* sp. The wine was found to contain a concentration of volatile acidity of 1.9 g/L, and the results of microbiological analysis of the wine indicated that it contained viable rod-shaped bacteria, suspected to be *Acetobacter* sp., as well as viable lactic acid bacteria and yeast (see also Institute publication #718).

Observations of the linings of the screwcaps showed perforated rings around the area of contact with the finish of the bottles, with some 'puckering' of the liner material. Although the linings inside the caps of all of the bottles appeared to be damaged to some extent, 'tide lines' existed in only some of the bottles. These bottles were found to exhibit higher levels of volatile acidity, little or no free SO_2, and exhibited high turbidities compared to samples with no 'tide lines'. The wine in six bottles that did not exhibit 'tide lines' contained concentrations of volatile acidity of between 0.53 and 0.56 g/L. The wine in five other bottles that did exhibit 'tide lnes' ranged from 0.86 to 1.21 g/L. These observations suggest that some of the closures had apparently been damaged to such an extent that they had allowed air to enter those bottles. This had resulted in the loss of SO_2, thus allowing the growth of spoilage microorganisms that had produced the elevated concentrations of acetic acid, and caused the increase in turbidity and creation of the 'tide lines' in those bottles.

A workshop to be staged at the 12th Australian Wine Industry Technical Conference, which will be held in Melbourne in July 2004, will examine factors relating to the correct application, and quality control of screwcap closures.

Investigations conducted into the nature and amelioration of taints in grapes and wine, caused by smoke resulting from bushfires

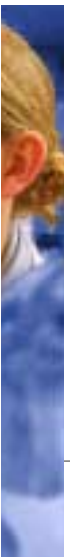
During the 2003 vintage, Industry Services staff spent a large amount of time dealing with the issue of 'smoky' taint in grapes and wine resulting from the bushfires that occurred in Victoria and southern New South Wales in January and February 2003. The involvement of the Institute's Analytical Service in expediting the analysis of samples as part of these investigations is acknowledged, as is the input of other Institute staff particularly Dr Leigh Francis, Kate Lattey, Dr Mark Sefton, Dr Alan Pollnitz, Dr Paul Henschke and Creina Stockley.

The Industry Services team considers that this issue was the single largest problem dealt with since the inception of the team, both in terms of its value and the numbers of wine companies and grapegrowers affected. The investigations began with approaches from technical staff of several companies in Victoria and South Australia, who considered that there was a problem with 'smoke taint' in fruit that they had either processed or had contracted to purchase. Over subsequent days the number of approaches to the Institute became overwhelming, and it was clear that a dedicated trial was required to properly understand the nature and extent of the problem, rather than a 'scatter gun approach' of working with several companies concurrently. The Institute, therefore, supported a move by the Alpine Valley Winemakers and Grapegrowers Association to fund a researcher to conduct various trials. The Industry Services team helped to design targeted trials, much of this work necessarily being performed over a very short time frame. It is considered that the approach taken by the association's nominees and other affected parties was extremely thorough and professional, such that well controlled and executed replicated experiments were conducted, and well prepared samples delivered to the Institute for analysis. The fact that the affected regions involved declared phylloxera zones complicated the preparation and freighting of samples to Adelaide, and at all times phylloxera control protocols were followed. The Institute also acknowledges the valuable input and assistance provided by John Whiting of the Victorian Department of Primary Industries, Jill Kuchel of Vignoble Monitoring Services, Wendy Cameron and Terry Barnett of Brown Brothers Milawa Vineyards, and Shayne Cunningham of Gapsted Wines. As a result of various trials, it is considered that a high level of understanding of the problem has been achieved, but unfortunately in spite of which, the problem has proved to be quite intractable in most cases.

The main conclusions of the initial investigations were:

> Various wines and juices submitted to the Institute were indeed considered to exhibit characters variously described by the Institute's sensory panel as smoky, burnt, ash, ashtray, salami, smoked salmon etc.

> It was established that guaiacol and 4-methylguaiacol were the most important compounds contributing to the sensory taint. Guaiacol and 4-methylguaiacol are compounds that commonly occur in wines that have been matured in contact with toasted oak products, and are formed during the toasting process from the degradation of lignin. Importantly, a back-palate *excessively drying* character and a lingering retro-nasal ash character appeared to be more pronounced in smoke-affected samples than in juices or wines spiked



with similar concentrations of guaiacol. **It is therefore considered likely that other compounds resulting from the smoke were present in the juices and wines at albeit very low concentrations. However, it was not possible with the resources available to identify the presence of such compounds, their possible concentrations, or their possible sensory or other impacts. As such, guaiacol and 4-methylguaiacol cannot be considered as solely responsible for the identified taint.**

> Samples of a number of reference juices obtained from The University of Adelaide's Hickinbotham Roseworthy Wine Science Laboratory (WSL), which had been sourced from various vineyards around Adelaide where there had not been bushfires during the growing season, were found to contain no detectable guaiacol or 4 methylguaiacol. The assistance of Mr Stephen Clarke in providing these samples is acknowledged. Mr Phil Spillman (pers. comm.) informed the Team Manager that in his trials previously conducted at the Institute using a Cabernet Sauvignon wine sourced from the 1993 vintage in Coonawarra, a control wine that was stored in stainless steel for 93 weeks contained 5 µg/L of guaiacol, which was attributed to the hydrolysis of fruit-derived precursors over time.

> The concentrations of guaiacol and 4-methylguaiacol in various sets of juice and wine samples were strongly correlated with the overall sensory panel rating of the intensity of the taint. **It is important to note, however, that this does not imply that guaiacol and 4-methylguaiacol are solely responsible for the taint, and no work was performed to investigate any other compound that might be present in affected juices or wines.**

> Little information is available in the literature concerning the sensory thresholds of guaiacol in juices and wines. The sensory difference threshold for guaiacol in white juice was established to be 6 µg/L or less, using a sensory panel comprised of tasters who had previously been exposed to albeit comparatively small numbers of affected samples. It is possible that tasters with more experience in the identification of the taint would have demonstrated a lower threshold.

Using the same tasting panel, the sensory difference threshold in a red wine that contained a background level of 37 µg/L of guaiacol was established to be between approximately 15 and 25 µg/L. It is possible that in a wine with a lower background concentration of guaiacol the sensory threshold would also be lower. Some of the bushfire smoke affected red wines analysed contained in excess of 70 µg/L of guaiacol. The Institute's Analytical Service database contains results of several hundred guaiacol analyses. This sample-set must be considered as potentially heavily skewed towards wooded wines and wines that formed part of various commercial cooperage trials. Approximately 60% of these samples contain guaiacol concentrations below 20 µg/L.

A trial was conducted to ascertain if it was possible to reduce the guaiacol concentration in grapes and wine by applying various 'vineyard-washing' treatments. The treatments examined were cold water, cold water plus wetting agent, warm water (approximately 25°C when contacting the fruit), cold water plus 5% ethanol, and milk. None of these treatments reduced the guaiacol concentration in either free run juice or crushed grapes that were macerated with skins for either one hour or 24 hours. Guaiacol concentrations were found to increase with increasing maceration time, and samples macerated with leaves contained higher guaiacol concentrations. **This finding supported empirical observations made by winemakers, that machine harvested fruit was more badly affected than similar fruit that had been hand picked, and further empirical observations that the free-run juice from whole bunch pressing was less badly affected than the pressings, or juice from similar fruit that had been machine harvested.** Samples of the liquids from each of the washing treatments were also collected and analysed. While it was obvious that some of them contained particulate matter, little or no guaiacol was detected in them. The Ovens Research Station (Department of Primary Industries [DPI] Victoria) examined bunches and leaves from the trial and ascertained that approximately 90% of ash and particulate matter had been washed off the grapes by the water washing treatments. As it is possible that this ash contained compounds with an undesirable sensory or oenological impact, it was deemed preferable to remove it before harvesting by applying a water spray in the vineyard.

These results from this initial vineyard washing trial were available within four days of the instigation of the trial, and based on them the following advice was developed:

> Leaf plucking, followed by a high-volume, high-pressure cold water wash in the vineyard, followed by hand picking and whole bunch pressing with the separation of juice into several press fractions, was most likely to minimise the taint and allow maximum value to be salvaged.

> In the event that hand picking was not an option, then leaf plucking followed by a high-volume, high-pressure cold water wash in the vineyard, and minimisation of leaf matter entering the fruit during harvesting should be employed.

This advice was actively disseminated to growers and wine companies via various grower and winemaker associations, DPI Victoria, and many individual contacts made with the Institute by growers and media outlets (see Table 13).

Investigations were also conducted in order to ascertain if it was possible to reduce or ameliorate the taint during winemaking. A current Institute research project is examining the ability of various fining agents to 'scalp' or remove various flavour compounds from wine, and a preliminary summary of the results by Institute Research Chemist, Dr Alan Pollnitz, was published in *Technical Review* issue 142 (February 2003), and is also discussed elsewhere in this report. Guaiacol was one of the flavour compounds examined. Of the fining agents examined, only activated carbon was found to remove any guaiacol, the concentration being reduced by approximately 5% with the addition of 300 mg/L of carbon. The effect of colloidal silica was not examined in the Pollnitz study, and consequently the action of this fining agent was tested on a smoke affected Sauvignon Blanc juice that had been found to contain 18 µg/L of guaiacol. Additions of 0.5 and 1 g/L colloidal silica were added using Bakesol 30, and the samples were shaken for two minutes, and then allowed to stand for one hour before being filtered and analysed for guaiacol concentration. The concentration of guaiacol was not affected by either fining rate. The intensity of smoky or ashy taint was not sensorially assessed in these samples.

It was considered that an explanation was required as to why the vineyard washing treatments had no effect on guaiacol and 4-methylguaiacol, compounds that are reported to be very soluble in water. It was considered important to understand the location of the guaiacol within or on the grape, in order to assist the minimisation of its extraction during processing. Therefore, various samples of grapes were peeled, and the skins and pulp were analysed separately. The results of this experiment were as follows:

> Guaiacol and 4-methylguaiacol were detected in all of the skin samples, but were not detected in any of the pulp samples.

> In subsequent experiments, washing bunches in 95% ethanol for 30 seconds had no effect on the concentration of guaiacol in crushed grape samples that were macerated with skins for 24 hours. The ethanol that had been used for washing subsequently contained a very low concentration of guaiacol equivalent to approximately 4 µg per bunch. Similar results were obtained from an experiment using hexane as the solvent, rather than ethanol.

One purpose of this experiment was to ascertain if the guaiacol was located within the wax bloom on the grapes, or whether it was partitioned in the skin. Based on the assumption that the ethanol and hexane did remove the wax bloom from the grapes, the results suggest that the guaiacol had permeated the grape skin, but that it had not passed through the skins into the grape pulp. It is suggested that this experiment should be repeated using chloroform, as previous workers have used this solvent in experiments to elucidate the composition of the wax cuticle on grape skins (Martin 1960, and Radler 1965).

Based on the knowledge that the guaiacol appeared to be located in the grape skin, it was considered reasonable to predict that the maximum extraction of the compound would occur during red wine maceration in the presence of ethanol.

A number of batches of red grapes were analysed for guaiacol concentration pre-crushing, using the 24-hour maceration treatment that had been used in earlier experiments. Additionally, samples were homogenized in a 10% ethanol solution in order to ascertain if this treatment could be used to predict, pre-processing, the total amount of guaiacol that would subsequently be extracted during fermentation. Fermentations were sampled daily and at pressing, where free run and light and heavy press fractions were sampled. Samples of marc ex-pressing were also analysed. The following results were obtained from these various red wine fermentation experiments:

> The concentration of guaiacol increased in a near linear manner for the first three to four days of fermentation, and increased only slightly thereafter.

> Free run, light and heavy press fractions all contained the same concentration of guaiacol and 4-methylguaiacol.

> Approximately 25-33% of the total guaiacol present in grapes was apparently extracted when grapes were crushed and macerated with skins for approximately 24 hours before guaiacol analysis was conducted.

> Approximately 75% of the total guaiacol present was apparently extracted from grape skins when they were homogenised in a 10% ethanol solution.

> A small amount of guaiacol and 4-methylguaiacol remained in the marc after pressing.

Conclusions

The Institute had previously been asked to consider if smoke resulting from controlled burning of bushland might result in a taint occurring in grapes in nearby vineyards, and had provided advice that there was a possibility that such a taint could arise. The results of this investigation provide conclusive evidence that tainting of grapes and wine by bushfire smoke can occur, and potentially have a major economic impact. Many affected white juices, wines and especially sparkling base wines, were deemed to be 'unfit for purpose' and were consequently severely downgraded in terms of value. It remains to be seen what the commercial impact of the elevated guaiacol levels in many red wines will be.

As Australian viticulture continues to spread into locations that might be considered as more bushfire-prone than many established grape growing areas, there is a possibility that smoke taint might become a sporadic but more common occurrence in the future. It is evident that few vineyards were actually damaged by the 2003 fires, but damage caused by smoke taint was widespread. It is, therefore, apparent that in this situation insurance cover for smoke damage would have been of greater benefit than for fire damage. However, while it appears that few growers had insurance cover for smoke damage, many do maintain insurance against contamination.

In spite of the fact that this project has greatly increased the understanding of the nature of this problem, solutions remain elusive. Late in the reporting period, the Institute analysed a number of commercial samples that were purported to originate from a reverse osmosis treatment of red wines, which was being applied with a view to reducing the intensity of the taint, and the concentration of guaiacol in the wines. The results demonstrated an apparent reduction in guaiacol concentration of approximately one third, in the two wines tested. Sensory analysis was conduced on pre- and post-treatment samples of one of the wines, with the Institute's sensory panel rating samples that contained lower guaiacol concentrations as lower in *smoke taint*. It is possible that the process may also remove compounds other than guaiacol that may contribute to the perception of the taint. However, there were no significant differences between the panel's ratings of *preference* for the samples pre- or post-treatment. While the results of this trial are encouraging and there could be merit in follow-up trials, the Institute advises people who are considering the use of this technology commercially to conduct their own trials, and to undertake rigorous sensory evaluation on samples pre- and post-treatment, and to have the samples independently analysed for guaiacol concentration.

References

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Winemaking consultation

The Industry Services team provides a winemaking consultancy service principally through the Manager, Peter Godden, a qualified and experienced winemaker; Adrian Coulter, a Graduate in the Diploma in Oenology from The University of Adelaide; and Mark Gishen, a Chemical Engineering graduate from The University of Melbourne with considerable experience in the Hunter Valley industry. Greg Ruediger, the Trace Analysis Laboratory Supervisor, works with the Industry Services team on a 0.25 basis, and also holds a Graduate Diploma in Oenology from The University of Adelaide. Both Adrian Coulter and Greg Ruediger have gained vintage experience with local wineries during recent years. Further technical advice is provided by Ella Robinson, who has a BA and BSc from The University of Adelaide, and Geoff Cowey, who has a BSc from The University of Adelaide.

Most queries received are technical in nature and arise predominantly from Australian winemakers. However, many queries are also received from wine industry suppliers and Government bodies, as well as a relatively small number from the general public and secondary and tertiary students. The number of calls received from journalists has increased substantially over a number of years (see Table 13 for list of media enquiries handled during the year), and this trend is considered to reflect both increasing public awareness and interest in the wine industry, and also the nature of the projects being pursued by the Industry Services team, particularly the ongoing investigation into the technical performance of wine closures, and more recently the issues relating to the potential spoilage yeast *Dekkera/Brettanomyces*. Investigations into the effect of bushfire smoke on grapes and wine also resulted in a number of media enquiries. The majority of queries are answered either by telephone, email or facsimile, and Industry Services staff supply approximately 500 technical papers or other pieces of relevant literature to callers each year, via the John Fornachon Memorial Library. Increasingly, Industry Services staff are also able to direct callers to web-based information, both on the Institute's own, and other web sites. Furthermore, the support facilities provided by other Institute research and library staff are important in

Table 8. Enquiries received by Industry Services advisory staff during the past three years*

	2000/01	2001/02	2002/03
Wineries	1155	1008	1184
Government organisations	117	101	97
Other	359	469	368
Students	26	31	27
Total	1657	1609	1676

*This does not include the calls to other senior Institute staff, e.g. the Viticulturist responds to over 400 enquiries and the Health and Regulatory Information Manager responds to over 300 enquiries each year.

supplying relevant information to callers, and the analytical capacity of the Industry Services Laboratory plays an important role in responding to many of these enquiries. Most of the investigations recorded in Table 7 result in a full written report being prepared for the client. These reports contain a large amount of technical information relating to the problem being investigated and are written in a way which seeks to explain the underlying causes of the problems encountered, and provide advice on how to prevent them re-occurring. The reports are often accompanied by a number of technical references relating to the area of investigation.

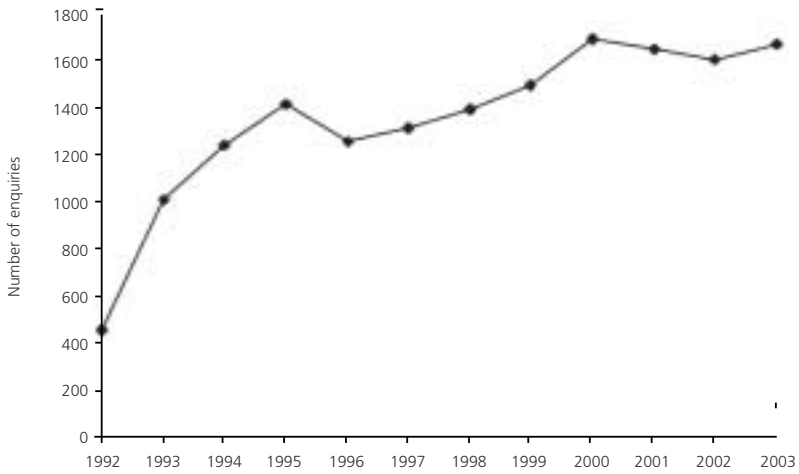

Figure 12. Enquiries received by advisory staff, 1992 to 2003

The Institute also often acts as a referral service, increasingly for Government bodies, wine industry suppliers and wine journalists, having links to Australian and international wine research and political bodies. The vast store of information, both formal (in the John Fornachon Memorial Library) and informal, is a valuable resource to the wider industry.

A summary of the enquiries received by Peter Godden, Adrian Coulter, Mark Gishen, Ella Robinson, Geoff Cowey and Greg Ruediger during 2002/2003 is presented in Table 8. The figures show a 4.2% increase in the number of enquiries received compared to the previous year. The proportion of requests received from wineries was the highest ever recorded at 71%. A six-monthly record of 980 calls was recorded during the first half of 2003, including 43 calls relating to the effects of bushfire smoke on grapes and wines. The requests recorded in the 'other' category in Table 8 are received largely from industry suppliers, and as reported in 2002, a substantial number of requests for technical information received from journalists (see Table 13), as discussed above.
The number of enquiries handled by the advisory service between 1992 and the current year is shown in Figure 12. To some degree, the increase reflects the expansion that has occurred in the wider Australian wine industry, and as such the growth has slowed in recent years. However, it is evident that the number of enquiries has stabilised to some extent at an historically high level, and indicates that a large number of personnel in the Australian wine industry regard the Institute as an important source of technical information. The Consultation and Investigative and Advisory Services are supported by Roadshow seminar and workshop tours, which are made on a rotating basis to 24 wine growing regions. Roadshows are generally organised in conjunction with local vignerons' associations, and are held in the second half of the calendar year. Whilst the Industry Services staff members are responsible for the organisation of these events, they rely heavily on input from all of the Institute's research teams, with six senior Institute staff members making twelve presentations in each full-day seminar. During the 24 months to the end of the 2002 Roadshow 'season', seminars were presented in 18 regions. During the current reporting period four states were visited (Western Australia, South Australia, Victoria and Tasmania), and ten seminars and five *Trouble free winemaking* workshops were presented (see Appendix 1).

Regional winemakers' and growers' associations are asked to select the presentations to be made at each seminar from a list of approximately 40 areas of current Institute activity, in order that seminars are closely tailored to the interests and needs of the audience. In addition to the formal presentations, Roadshows are also considered an important vehicle for the delivery of informal advice, and it is considered that if this contact were formally recorded then it would account for a substantial increase in the number of enquiries recorded in Table 8. The most common areas of informal discussion on recent Roadshows have been *Dekkera/Brettanomyces* and other microbiological stability issues, red wine phenolics, salinity both in terms of soil and vineyard effects and effect on wine, stuck fermentation, and the use of non-conventional yeasts.

The sixteenth Advanced Wine Assessment Course was held in July 2002, giving another thirty participants the opportunity to develop and test their sensory evaluation performance. This was the fourth course presented using a four-day format, which includes over 40 hours of activities over the four days, and 14 leading wine show judges, journalists and winemakers assisted in the presentation of the course. The four-day course has proved to be very successful, and demand for places in the course has increased substantially since the format was changed from the previous three days. The course content is continually being enhanced, and the sixteenth course included a number of new tasting and discussion exercises that were intended to simulate the dynamics associated with judging as part of a wine show panel. Extremely positive written feedback regarding the course was received from a number of participants and guest judges. As in the past, some Associate Judges for the 2002 Adelaide Wine Show and other shows were selected from the most successful recent participants in the Course. Similar approaches from other wine shows are encouraged.

During the reporting period, the team Manager had substantial involvement in the planning of the 12th Australian Wine Industry Technical Conference, which will be held in Melbourne in July 2004, serving on both the Planning and Program Committees, and has primary responsibility for coordinating the staging of approximately 70 workshops for the Conference. Industry Services team members are also regular contributors to the Institute's *Technical Review*, periodically provide presentations for external seminars and conferences (see Appendix 1), and also provided eleven hours of lectures to Oenology students at the University of Adelaide, and other lectures to students from La Trobe University's Wodonga Campus (see Appendix 2), during the current reporting year.

Evaluation of new analytical techniques and of processing aids for winemaking

Staff: Peter Godden, Adrian Coulter, Mark Gishen, Ella Robinson, Dr Mark Sefton, Dr Alan Pollnitz, Dimitra Capone, Geoff Cowey, Greg Ruediger, Trudy Wallis and Peter Valente

The Industry Services Laboratory maintains a GWRDC-funded project for the improvement, development and evaluation of methods of wine analysis, and the evaluation of winemaking processing aids and additives. The evaluations take one of two forms: the relative performance of commercially available products and the evaluation of new materials marketed to the industry. Industry Services staff also provide advice to the Institute's Analytical Service on the development of protocols which relate to trials being conducted on a fee for service basis for various wine companies and industry suppliers. This project ceased on 30 June 2003, and many of its functions will be incorporated into

a new project *Application of research and development to industry problems and opportunities*. The first subject to be addressed by the new project will be *Investigations into the relationship between* Dekkera/Brettanomyces *yeast and wine spoilage*, thus during the current reporting year, many of the resources of this project were directed toward preliminary research and methods development associated with the *Dekkera/Brettanomyces* investigations.

As previously reported, during the preceding three years the vast majority of resources allocated to this project were used for the Closure Trial, a wide-ranging trial that is examining the technical performance of 14 different closure types. Testing in this project continues, albeit at a lower level of intensity than in previous years, and results of the trial, particularly those associated with the measurement of sulfur dioxide (SO_2) concentration and wine browning as measured by optical density at 420 nanometers (OD_{420}), and sensory evaluation of wine sealed with each of the closures, continue to be published periodically. During the current year results were published in *Technical Review* issue #139[1] (August 2002), and the Proceedings of the Romeo Bragato Conference[1], Christchurch, New Zealand, September 2002. The trial continues to attract interest from both industry and mainstream media (see Table 13), and in a letter to the Institute from a representative of a leading UK supermarket chain the trial was described as a "catalyst for the changes in closure use that are now apparent".

Note: The 'closure trial' was not designed to be a survey of closures available in the marketplace, and not all of the closures available at the time the trial commenced were included. Readers should also note that the currently available stocks of some of the closures examined might differ from those available when the trial was implemented, and that the results reported represent the performance of each closure when used to seal only one wine, under the conditions defined. Care should therefore be exercised in relating the results to other wine types, or to wines bottled and stored under different conditions. It should also be noted that some closures may be suitable for use in products with a short shelf life, and may perform well over a particular time span, but less well over a longer term. Winemakers therefore need to assess the anticipated shelf life of the wine to be bottled when making decisions on closure use.

The closures that are being examined in this study comprise a roll-on tamper-evident (ROTE) screw-cap closure, two grades of natural conventional cork (reference 2, 44 mm length and reference 3, 38 mm length), two 'technical cork' closures (cork-based closures that also contain a synthetic component: Sabaté 'Altec' and Amorim One plus One 'Twintop'), and nine closures manufactured from synthetic materials, three of which are produced by extrusion processes (ECORC, Nomacorc and NuKorc) and six by moulding (Aegis, Auscork, Betacorque, Supremecorq, Californian 'Tage'[1] and Integra). Various aspects of the performance of the closures are being examined, including those relating to each closure's physical characteristics and extraction from the bottle, chemical analysis of the wine in order to examine apparent sealing performance, and sensory analysis.

The retention of SO_2 in wine sealed with each of the closures continues to be a pivotal measurement in this investigation. Loss of SO_2, presumably due to the degree of oxygen ingress allowed by the closure, has been found to correlate well with the development of brown colour in the wine, and also the rating of oxidised character during sensory evaluation, at all testing intervals. The concentrations of free SO_2 recorded in wine sealed with each of the closures at six months, were also found to be excellent predictors of free SO_2 concentration and browning and rating of oxidised aroma in the wine after 24 months, and to a lesser extent after 36 months. The fourth anniversary of bottling the trial wine occurred late in the reporting period, and at this point wine sealed with only five closures: the 'ROTE' roll-on tamper-evident 'screw cap', the Altec and One plus One technical corks, and the reference 2 and reference 3 corks, were tested for SO_2 concentration, OD_{420} using conventional cuvette measurements (Table 9), and sensory analysis for aroma only. Additionally, wine sealed with all 14 closures was tested for OD_{420} using a non-destructive in-bottle measurement developed by Skouroumounis et. al. (Figure 13) (Institute publication #731).

Table 9. Mean SO_2 concentration and optical density at 420 nanometers (OD_{420}) following 48 months of storage in an inverted position

		48 months inverted storage (n = 20)[a]	OD_{420} (cuvette measurements) (au)[c] (n = 20)[a]
Altec	Free	14 *(2)*[b]	0.156 *(0.003)*
	Total	79 *(3)*	
One plus One	Free	9 *(2)*	0.184 *(0.007)*
	Total	70 *(3)*	
Reference 2, 44 mm cork	Free	6 *(4)*	0.194 *(0.018)*
	Total	62 *(11)*	
Reference 3, 38 mm cork	Free	5 *(4)*	0.205 *(0.016)*
	Total	56 *(12)*	
ROTE	Free	15 *(4)*	0.163 *(0.011)*
	Total	81 *(5)*	

[a]n = 18 for ROTE, [b]figures in parentheses are standard deviations, [c]au = absorbance units

Data presented in Table 9 indicate that the important trends observed during earlier testing have continued up to the four-year post-bottling time point, with regard to the five closures tested. In particular, wine sealed with the ROTE closure continues to retain the greatest concentration of SO_2, but the concentration was not significantly different to that in wine sealed with the Altec closure. Wine sealed with the Altec closure demonstrated a slightly lower level of brown colour development than wine sealed with the ROTE closure at the four-year post-bottling time point, as measured by the conventional cuvette method, but this was reversed in readings made using the in-bottle test. However, it is clear that differences among readings made using the two methods are minimal, and in general the measurement of brown colour by the non-destructive test (Figure 13) matches the cuvette measurements extremely closely for the five closures tested at the four-year post bottling time point (for further documentation of this measurement, see Institute publication #731). It is equally apparent that the separation of closures on the basis of brown colour in the wine as measured by both tests, is closely related to the concentration of SO_2 retained in wine sealed with each of the closures at 48-months post bottling. Additionally, it is clear that small differences in SO_2 concentration identified as early as the six-month post bottling time point, relate strongly to increasingly large differences in brown colour in wine sealed with all of the closures, as the trial progresses.

[1]The Institute wishes to make it clear that any reference to 'Tage' closures in these publications, and also in this publication, are references to closures that were supplied by Esvin Wine Resources, Auckland, New Zealand in May 1999, and not to closures supplied by Novembal. The Institute has been informed that the closures being examined were made by a Californian manufacturer, and not by Novembal, and Novembal has informed the Institute that these closures are not the same as Tage closures now being made by Novembal. The Institute takes no position on Esvin's right, or that of the Californian manufacturer, to make or supply closures under the name 'Tage'.

Flavour scalping sub-project

It has long been recognised that wine bottle closures play a role in the way wine flavour changes during bottle storage, principally by acting as a partial barrier to the transmission of oxygen. The occasional extraction of 2,4,6-trichloroanisole (TCA) from cork bark closures into wine is well known as a means whereby an unwanted musty aroma can be imparted to wine. There is some evidence that other constituents of closures can also be extracted into wine with various effects on wine aroma and flavour.

The impact of wine bottle closures on wine aroma and flavour is generally thought of in terms of these processes. However, it is well known that packaging components in other food products can also modify product quality by absorbing aroma compounds from the product, a process sometimes known as 'flavour scalping'. Such absorption could, therefore, also take place with wine bottle closures during bottle storage. Recently we observed a case where red wines, contaminated with the musty-smelling compound 2,3,4,6-tetrachloroanisole (TeCA) during barrel maturation, were effectively 'decontaminated' as a result of the cork closures absorbing the TeCA from the wine during bottle storage (Institute publication #616). In this instance, the absorption phenomenon clearly resulted in an improvement in wine quality. This capacity to absorb aroma active compounds from wine was also demonstrated for 'bag in box' packaging materials (Institute publication #702).

A long term trial was set up several years ago (by Dr Mark Sefton, Dimitra Capone and others) to study the absorptive capacity (flavour scalping) of various wine bottle closures. The closures used in this trial were from the same batches used in the Institute's earlier closure trial (bottling in May 1999) which focussed on white wine oxidation and sensory properties (Institute publications #666, 671, 675, 676, and 677). The closures used in this 'scalping' trial were a roll-on tamper-evident screw cap (ROTE), two grades of natural bark cork (Reference 2 and 3), a technical cork closure, and seven synthetic closures (a preliminary report on this was given in *Technical Review* issue 144, June 2003).

Absorption of flavour compounds can be determined in one of two ways — either directly by extracting the closures themselves and analysing the extracts for absorbed compounds, or, indirectly, by determining the decrease in concentration of these compounds in wine during storage. For technical reasons, the latter option is far more practical and was the approach taken in this study. In this case it was also essential to have control samples stored with and without air in all-glass containers (sealed ampoules), so that the loss of wine components by chemical degradation could be distinguished from loss by absorption.

To observe the effects of bottle closures on a range of flavour compounds, a Semillon wine was spiked with each of the volatile compounds we wished to study. The wine was then bottled, the bottles sealed with the closures listed above and kept in an inverted position in a constant temperature storage facility. After two years of bottle storage, the concentration of some, but not all of the flavour compounds added to the wine changed significantly. Some of these changes can be attributed to absorption by the closures, others to chemical modification which took place regardless of how the wine was sealed.

In no case was there any evidence of absorption of any of the flavour compounds from the wine sealed under the ROTE closure. Where the concentration of compounds had decreased during the storage period, similar decreases in concentration was observed for control samples sealed in glass ampoules. Neither isobutylmethoxypyrazine nor the oak-related components guaiacol, 4-methylguaiacol, vanillin, *cis*- and *trans*-oak lactone, 4-ethylphenol and 4-ethylguaiacol changed concentration significantly in either the controls or bottled wines over the two year period. All of these compounds are therefore chemically stable in wine (including control wines stored under air) and are not affected by bottle closures.

The concentration of the simple fermentation esters changed in the control wines as a result of competing hydrolysis and esterification reactions in the wine. Some esters increased in concentration while others decreased. These changes cannot be extrapolated from this wine to others, because the factors affecting such changes (relative starting concentrations of esters and their corresponding acids) varies from one wine to another. Some esters were also affected by the closures (other than ROTE) to varying degrees. The short chain ethyl isobutyrate, ethyl butyrate and ethyl isovalerate were unaffected by the closures. Ethyl hexanoate, ethyl octanoate and ethyl decanoate were all partially absorbed by the closures, and for each closure type, the amount of absorption increased with increasing ester chain length.

Both natural closure types (Reference 2 and 3 corks) absorbed no ethyl hexanoate, 5% of ethyl octanoate and 20% of ethyl decanoate. The technical closure absorbed slightly more of these last two compounds (10% and 35% respectively). The synthetic closures showed significantly greater absorption of these esters. For ethyl hexanoate the absorption was slight, ranging from 5% (in three closures) to 15% (in two closures). Ethyl octanoate absorption varied from 30% (two closures) to 50% (one closure), and ethyl decanoate from 50% (two closures) to 70% (one closure).

There appeared to be little effect of the closure type on the concentration of the monoterpenes linalool, geraniol and nerol. The overwhelming factor affecting the concentration of these compounds in the wines was chemical degradation. At wine pH, nerol and geraniol are converted mainly to linalool which is in turn converted to the relatively weak-smelling alpha terpineol and terpin hydrate. In these trial wines, less than 5% of the added nerol and geraniol and 10% of the added linalool remained after two years. In the control samples there was some effect of headspace. Control wines stored under air had 30% less linalool than did wines stored under nitrogen.

The monoterpene rose oxide, which gives a 'lychee' character to some white wines, was partially absorbed by the synthetic closures only. This absorption ranged from 15% to 45%.

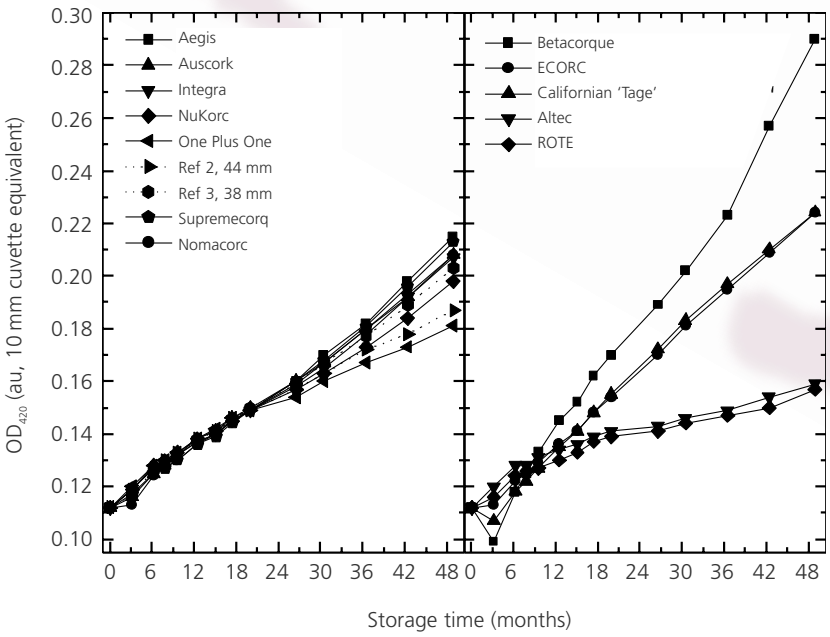

Figure 13. In-bottle, mean development of brown colour in wine sealed with each closure type, stored in an inverted position, as measured by optical density at 420 nm (OD_{420}). (n = 12 for all closures to 36 months, n = 9 for ref 3, and One plus One and n = 8 for ref 2 from 42 months onwards)

The taint compound naphthalene (added to give an idea of the absorption of structurally similar compounds) was substantially absorbed, particularly by the synthetic closures. The least absorptive of these removed 75%, while 90% was removed by the most absorptive. The natural and technical corks absorbed less of this compound (30% and 40%) respectively.

The grape derived hydrocarbon known as TDN can give a kerosene-like flavour to some white wines. It can sometimes be found in relatively high concentrations in Riesling wines, particularly those that have been in bottle for two years or more. This compound was the most strongly affected by the closures. All of the synthetic closures had removed between 96% and 98% of the TDN after two years. The natural corks and technical closures removed 50% and 70% respectively, while the ROTE closures removed none at all. TDN is sometimes regarded as undesirable when present in high quantities, so the capacity of closures to remove this particular flavour compound (and perhaps others) from wine is certainly not always deleterious to wine quality.

In summary, relatively non-polar volatile compounds can be absorbed by synthetic and natural cork closures, but not by ROTE screwcaps. Synthetic closures showed a much greater capacity to absorb these non-polar compounds than did natural cork bark or technical closures. The technical closure behaved in a similar fashion to the natural corks but had a slightly higher capacity for absorption. Among the synthetic closures, some consistently appeared to have had a higher capacity for absorption than others. Some compounds (oak components, isobutylmethoxypyrazine, 4-ethylphenol, TDN) are completely stable in wine, even with an air headspace, while others (monoterpenes, damascenone) are substantially degraded in wine whatever the closure.

Further data analyses and interpretation of the data from this study are currently underway. At this point in time, it appears clear that the choice of closure may have a dramatic effect on wine sensory characteristics not only due to gross oxidative changes and transfer of taint compounds described earlier (e.g. Institute publication #666) but also through direct absorption of compounds to the closure material. As closure manufacturing technologies and materials are likely to change due to ongoing commercial developments, winemakers must at all times conduct their own trials to make informed choices about the closures most suited to their preferred styles of wine and commercial objectives.

Flavour absorption by fining agents

An Honours student, Matthew Clarke, under the supervision of Dr Alan Pollnitz, has recently completed a project on the effect of fining treatments on the concentration of aroma compounds in a Chardonnay wine. The fining agents examined were bentonite, PVPP, egg albumin, casein, gelatin, isinglass, activated carbon (charcoal) and copper sulfate.

Even the mildest fining removed more than 20% of every ester analysed. Generally, the shorter chain, more polar, more volatile esters were more affected by the fining agents, presumably due to their stronger hydrogen bonding, a mode of action attributed to some of these fining agents. This trend is the opposite to that observed in the 'flavour scalping' trial reported above where it is likely that the hydrophobicity of the longer chain esters makes them more prone to adsorption out of the wine. Of the monoterpene alcohols, linalool, nerol, geraniol and alpha-terpineol, only linalool was significantly affected by fining by agents other than carbon, which removed approx. 10% of all four compounds. The loss of linalool ranged from 5% due to isinglass up to 18% due to PVPP.

Damascenone and beta-ionone were unaffected by fining, except by bentonite and copper sulfate which removed about 5%, PVPP which removed 10%, and carbon which removed almost 20%, of both compounds. The oak compounds were little affected by fining. The largest effects observed were from carbon, which removed only up to 9% of the oak compounds.

Naphthalene and TDN behaved in a similar manner to each other, with about 10% more TDN being removed (compared to naphthalene) in every case. The extent of removal of TDN ranged from 30% for isinglass up to 70% for carbon.

Fining has the potential to affect the aroma and flavour profile of wine. The choice of fining agent is important, as not all compounds are affected in the same way. Note that the Institute wishes to reiterate that fining is a very important part of winemaking and that the inevitable scalping that takes place during fining should not be interpreted as an argument for doing away with fining.

Analytical method development and evaluation

The majority of the methods development work that has been performed during the current year relates to the investigations into the relationships between *Dekkera/Brettanomyces* spoilage yeast and wine.

The development of a GC-MS method for the quantification of isovaleric acid (3-methyl butyric acid) in wine, using a deuterated standard of isovaleric acid that was produced at the Institute under the GWRDC-funded project AWR *6 Grape Composition and Wine Flavour*, was reported in the previous Annual Report. Isovaleric acid is reported in the literature as an important spoilage compound produced by *Dekkera/Brettanomyces* yeast. During the current year it was ascertained that during GC-MS analysis of isovaleric acid, a second acid, 2-methylbutyric acid, was eluting at the same time. Subsequently, Geoff Cowey of the Industry Services team worked with Dimitra Capone under project AWR 6, to develop a GC-MS method for the determination of the concentration of both 3 methylbutyric, and 2-methylbutyric acids in wine.

Also in response to the requirements of the *Dekkera/Brettanomyces* investigations, development of HPLC methods for the quantification of coumaric and ferulic acids in both red and white wines and grape homogenates has continued. Coumaric and ferulic acids are precursors for the production of the spoilage compounds 4-ethylphenol and 4-ethylguaicol by *Dekkera/Brettanomyces* yeast, and these methods are considered crucial for the progression of the *Dekkera/Brettanomyces* investigations, allowing the development and subsequent degradation of the precursors to be measured during fruit ripening, winemaking and subsequent wine storage.

Further methods that are likely to greatly enhance the Institute's investigative capabilities in future years, have also been developed during the current reporting period by the Institute's Mass Spectrometry facility manager Mr Yoji Hayasaka and technical officer Ms Gayle Baldock, and are discussed elsewhere in this report.

Assistance and advice were provided to the Analytical Service by reviewing method validation reports for conformance with the requirements of the Institute's quality system, which is based on NATA requirements and ISO Guide 25. This work is conducted by the Institute's Quality Liaison Manager, Mark Gishen, who is part of the Industry Services team, and the following methods were reviewed during the year: Tetraconazole and cyproconazole in wine, juice, grapes and marc by GC-MS; Quinoxyfen in wine, juice, grapes and marc by GC-MS; Histamine and tyramine in red and white wine by HPLC; Fludioxonil, pyrimethanil, fenhexamid, tebufenozide and cyprodinil in juice wine and grapes by HPLC; Isovaleric acid in red and white wine by GC-MS; Emamectin in juice, wine, grapes and marc by HPLC. In addition, NATA accreditation was obtained for the methods: Malathion and Fenitrothion in wine and juice.

Targeted training of wine industry personnel: compilation of a technical reference manual and delivery of complementary workshops.

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The primary aim of this project is to produce flexible and updateable information packages on selected technical subjects, which will be delivered to wine grape levy payers via the internet, and workshops to be held in all major grape growing areas of Australia in conjunction with the existing Roadshow seminars.

The Institute has a vast amount of collective knowledge pertaining to grape and wine production, much of it generated by over 48 years of research and through Industry Services' projects at the Institute, that have been supported by Australian winemakers and grapegrowers. Although this research has produced in excess of 730 technical publications, there is also a great deal of information generated and recorded in a more informal manner by the staff concerned. In addition, a great deal of data pertaining to the composition of Australian wine is stored in various databases at the Institute, some of which have been in existence for several decades. Collectively, this information is a resource of great potential value to the Australian wine industry. This project, therefore, seeks to make a record of this information, so that it can be delivered to the industry in a manner in which it is both readily useable, and relevant to those involved in day-to-day wine production. It is envisaged that the project will be ongoing, and that many areas of interest to winery technical personnel will be addressed in due course.

The information collated to date has been posted to the Institute's web site over the past two years by Industry Services staff. Australian winemakers and grapegrowers can access the information at http://www.awri.com.au/practical_solutions/ (or alternatively, visit the Institute's website at www.awri.com.au, and select 'Practical Solutions' followed by 'Hazes and Deposits'). This section of the site is password protected (the password has been previously supplied to Australian wineries). Australian grape growers and winemakers who require the password and who can confirm that they pay the Wine Grapes or Wine Research Levy, can obtain the password from the Institute's Librarian (Catherine Daniel on email: Catherine.Daniel@awri.com.au). However, the 'Industry Development and Support' section of the Institute's website also contains a great deal of other technical information, which is readily accessible by interested parties.

New material is periodically added to the site, and the existing information is enhanced. During the current year, in conjunction with the Communications and Publicity Manager, all the material on the 'hazes and deposits' section of the site has been edited and in some cases re-formatted, in order to enhance its functionality. New digital images of deposits isolated from wine have also been added.

As discussed elsewhere in this report the combined and related areas of hazes and deposits and microbiological instabilities continue to represent a significant proportion of the problem wine sample investigations conducted by the Industry Services team. In many cases it is apparent that the problems could have been avoided if winemakers were more aware of the causes of many types of instability, and were able to access practical and relevant tools with which to trouble-shoot the problems, before significant wine spoilage had occurred. The website and workshops address these issues, providing practical trouble-shooting information and simple diagnostic tests, with which to isolate and identify a wide range of hazes and deposits in wine.

During the reporting year, five *Trouble-free winemaking – identification and management of common wine instabilities* workshops were staged under the auspices of this project, in three states (see Appendix 1). The content of this workshop was further enhanced during the year in order to focus more strongly on helping winemakers understand the causes of common wine instabilities. In addition to discussing options for formal quality management systems, the workshops contain practical advice on the most efficient way to incorporate routine analytical testing into quality management programs, and suggestions on the most efficient way to use the information generated to identify where problems exist, or to identify potential problems before they occur. A recurring aim and theme of the workshop is to foster an understanding of how wine compositional variables are linked, such that a change in one variable, whether intended or not, can have a potentially dramatic flow-on effect leading to the occurrence of an instability.

Such a winemaking change may be as simple as picking fruit one degree Baumé riper than in previous years. The resulting higher alcohol concentration in the wines, all other things being equal, may lead to fermentation problems with resultant increase in residual sugar concentrations. These wines may take longer to complete malolactic fermentation (MLF) due to the elevated alcohol concentration, allowing the proliferation of unwanted microflora. Pre- or post-MLF growth of unwanted microflora, including *Dekkera/Brettanomyces* as discussed below, will be favoured by the availability of residual sugars, and such growth may directly or indirectly lead to the binding of free SO_2. This loss of SO_2 may in turn leave the wine more prone to oxidation and further microbiological activity, which in turn may lead to the formation of a deposit in the wine. The Industry Services staff considers that this scenario is becoming more common, and is apparent in both in requests for technical assistance received from industry, and in the nature of the problem solving investigations conducted. Increasingly, data contained in the Institute's various databases is being collated under the auspices of this project in order to enhance the content of the workshops, and to monitor various compositional trends in Australian wine. It is apparent that these data may be used to explain the number of wines investigated that exhibit the type of instability problem discussed above. As an example, Figure 14's left and centre panels plot the median and mean ethanol concentrations of Australian red wines recorded in the commercial Analytical Service database, by vintage between 1984 and 2002. Figure 14's right panel provides the numbers of samples from each vintage analysed. Whilst these data represent a cross section of Australian wines by value, they are probably skewed towards relatively higher rather than lower value wines. The data demonstrate a

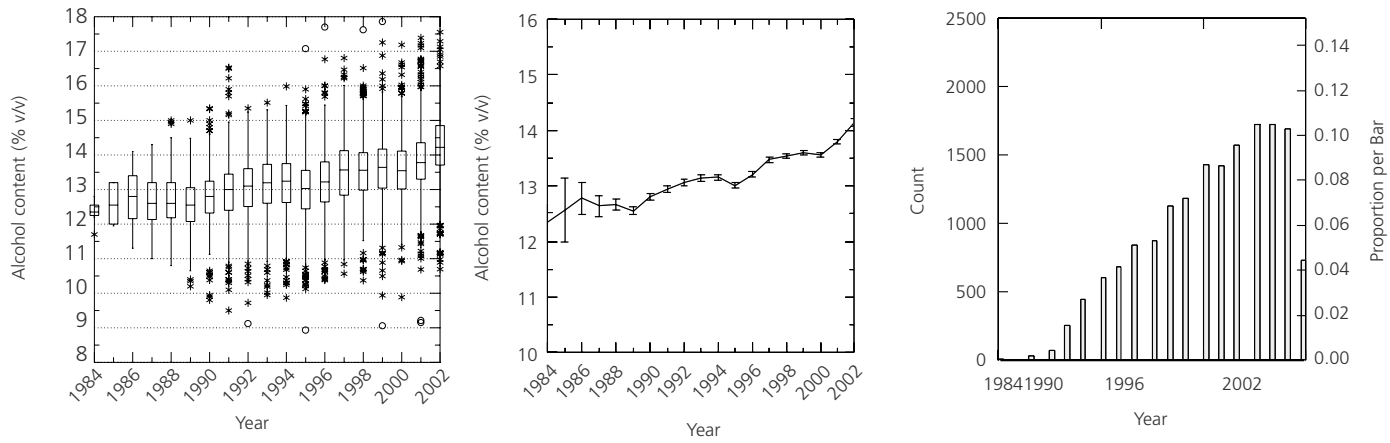

Figure 14. Median (left panel) and mean (centre panel) ethanol content of Australian red wines by vintage, 1984 to 2002, and numbers of samples analysed (right panel) (Source: The Australian Wine Research Institute Analytical Service database)

steady increase in ethanol content over this period, from a mean of approximately 12.6% in 1983, to a mean of approximately 14.2% in wines from the 2002 vintage. Interestingly, the rate of increase in mean ethanol concentration appears to have accelerated in 2001 and 2002, and in 2002 the median upper quartile of ethanol concentrations comprises wines that contain between approximately 14.9% and 16.5% ethanol. By consulting Figure 14's right hand panel, it can be ascertained that this quartile contains approximately 400 individual wines. It appears plausible that trends such as these are contributing to the number of Australian wines in which substantial potential quality could be lost during processing. This quality loss may be manifested in a diverse range of symptoms ranging from incomplete primary fermentation to subsequent microbiological instabilities causing deterioration of the sensory characteristics of wine, and the formation of hazes and deposits, as described above. The continued and enhanced targeting of Industry Services activities to foster industry wide understanding of these types of issues is therefore considered crucial in preventing quality loss, with consequent substantial cost savings for the Australian wine industry. Of particular interest in this respect is the finding by Helmut Guth and colleagues that high alcohol concentrations can attenuate the impact of volatile wine aroma compounds significantly (Guth and Sies 2002). Ironically therefore, the quest to accumulate high concentrations of aroma and flavour compounds in wine by getting very ripe fruit could well be counteracted by the parallel increase in ethanol content of the final wine. Clearly the industry is cognisant of this and therefore appear very supportive of the Institute's extremely challenging quest to seek 'low alcohol yeast' (see for example Institute publication #684).

Reference:

Guth, H.; Sies, A. (2002) Flavour of wines: towards an understanding by reconstitution experiments and an analysis of ethanol's effect on odour activity of key compounds. Blair, R.J.; Williams, P.J.; Høj, P.B. (eds.) Proceedings of the Eleventh Australian Wine Industry Technical Conference 7-11 October 2001. Adelaide, South Australia. 128-139.

Provision of technical information

Staff: Rae Blair, Catherine Daniel, Ingrid Oats, Melissa Francis

The John Fornachon Memorial Library holds the largest collection of wine technical literature in Australia. The Library's principal responsibility is to provide technical information to the Australian wine industry and to the researchers of the Institute. The Library is also used extensively by other groups such as students, institutional researchers, government bodies and private companies (see Table 10 for requests for information serviced during the year).

Information and document delivery services

The Library has excellent access to international databases, particularly in the fields of science, technology and medicine. If requested, the Librarian, Catherine Daniel, will carry out online searches on commercial databases on any appropriate topic (on a fee-for-service basis). Alternatively, Library staff can provide, free of charge, a report of relevant articles indexed on the Library's in-house databases.

The Library continues to enjoy strong support for all its services with increases in requests over that serviced in 2001/2002 (Table 10). During the year, the Library serviced a 24% increase in information requests from industry personnel and a 19% increase in requests from Institute staff. The number of Institute publications forwarded rose by 24% over the previous year's 33% increase, while requests for articles from Technical Review *rose by 19% over the previous year. The Library staff further handled a significant increase in requests for interlibrary loans from the Library's collection during the year. Industry's increased awareness and use of the Institute's web-accessible database has also contributed to an increase in enquiries, and this important service continues to be a valuable way for industry to access its technical literature, 24 hours per day, 7 days per week.*

Document delivery

The Library can supply either books or photocopies from its collection or obtain such items for wine industry clients through the interlibrary system. Patents or standards can also be ordered. Electronic ordering and delivery services mean that most interlibrary requests are fulfilled within five days. Charges apply for the supply of some items.

Specialised information services

The Library staff continue to be actively involved in the production of specialised information products for the benefit of the wine industry, such as the annual and web-based editions of the *Agrochemicals registered for use in Australian viticulture*, the bimonthly *Technical Review*, and several in-house technical information databases.

Library collection

A total of 74 monographs and 20 conference proceedings and over 3,600 new records were added to the library databases during the year. Further to this, 9,011 records sourced from the International Food Information Service (IFIS) were also added to the library databases during this period. Over 70% of these fully abstracted records replaced existing library database records where only the citation was available, thus enhancing the usefulness of information the library indexes on its databases.

Following a rigorous review of the library's in-house database, the total number of records indexed in the database fell slightly, although the number of unique records has in fact increased.

The Library subscribes to 54 journals and receives approximately 70 annual reports, journals and newsletters through exchange and donation. The Library also maintains a collection of over 22,000 reprints.

Table 10. Summary of information requests during 2002/2003

	Wine industry		Staff		Other[6]		Total		% change
	2003	2002	2003	2002	2003	2002	2003	2002	2003
Information requests	1081	869	943	792	1584	1702	3608	3363	7%
Interlibrary loans:									
> requests sent[1]	29	182	505	345			534	527	1%
> requests received[2]							88	53	66%
Technical Review requests[3]							230	193	19%
Technical Review articles forwarded[4]							1179	970	14%
Articles forwarded[5]							1424	1031	28%
Number of Institute publications forwarded							725	585	24%
Articles photocopied in JFML							3874	3740	4%

1 Staff at the JFML sent a request to another library for an article.
2 Requests received by the JFML from other libraries for articles from our collection.
3 Number of requests received for articles published in the *Technical Review*.
4 Number of articles forwarded (usually more than one article is requested).
5 Number of other articles forwarded, excluding staff publications (usually more than one article is requested).
6 90% of 'other' requests come from students and Government sources.

Library databases

A single search screen provides access to the Library's collection of over 43,000 books, conference proceedings, scientific, technical and medical reprint articles which are indexed on the Library's database catalogue; the bibliographic details of the Library's collection of the European Union wine legislation and details of the library's journal holdings are maintained on separate in-house databases.

Table 11. Number of records on the Library's catalogue, information and web-accessible databases

	No. of records
Library catalogue databases	
AWRI_Database: Books, conference proceedings, Theses, scientific and medical papers	43,282
JOURNALS: journals, newsletters, statistics and annual reports	404
Library information databases	
REGS: European Community wine legislation	393
ISYS – full text retrieval database covering	
United States of America Federal Register	877
Web accessible database (with searchable abstracts)	25,406

The Librarian provides reports, either on particular subjects or authors, listing the records retrieved from any of the Library's in-house databases. A summary of the size of the Library's catalogue and information databases is given in Table 11.

The Library provides access to its databases via the internet to Australian winemakers and grapegrowers paying the Primary Industries Excise Levy Tax 1999. The restriction in access is enforced to comply with copyright approvals obtained from the various publishers whose journals are the source of the abstracts that are accessible via the database. Library staff continue to edit database records to post onto the Library's web database, on an ongoing basis.

Agrochemicals Grid

As reported elsewhere in this Annual Report, Dr Sally Bell and Catherine Daniel prepared the thirteenth edition of the *Agrochemicals registered for use in Australian viticulture*. All levy payers receive a printed copy of the revised edition automatically, and the web-based edition (http://www.awri.com.au/agrochemicals/) is updated on a regular basis.

Technical Review

Technical Review is received by all Wine Grapes and Wine Research Levy paying wineries in Australia and, through subscription, by government and other organisations and individuals, both in Australia and overseas. *Technical Review* provides progress reports to the industry on the Institute's research as well as updates on relevant conferences, regulatory amendments and medical issues. *Technical Review's* 'Current Literature' section provides citation details and abstracts of recently published technical and scientific articles. Recipients of *Technical Review* may order articles featured in the 'Current Literature' section via a request form available within each issue. Restricted password access to *Technical Review* is also available on the Institute's website (http://www.awri.com.au/technical_review/latest_issue/). Dr Barbara Hardy AO and her sons continue to support the publication of *Technical Review* through regular generous financial contribution to the Thomas Walter Hardy Memorial Trust, and their ongoing support is gratefully acknowledged.

The collection of the 2002/2003 issues of *Technical Review* has been made available via a CD ROM and distributed free of charge to Wine Grapes and Wine Research Levy paying individuals. A simple search mechanism within the CD ROM facilitates fast access to technical notes, current literature abstracts and other matters of interest published throughout the year within the six issues.

Email service

The Email Advice and Information on Technical Issues Bulletin service continues to be a fast and cost-efficient way of disseminating important technical information to interested members of the Australian wine industry. There are 764 email addresses recorded to receive the email bulletins, and interested members of the Australian wine industry should submit their email address (to Rae.Blair@awri.com.au) should they wish to receive the email bulletins. Eleven email bulletins were issued during the year and are shown in Table 12.

The John Fornachon Memorial Library Endowment Fund

The Institute acts as the Trustee of this fund, which was established in 1969 by donations from winemakers and friends of the late John Fornachon, the first Director of Research of the Institute. The Library is funded by an annual grant from the Grape and Wine Research and Development Corporation, together with the income generated from investment of the Endowment Fund.

Acknowledgements

Donations of books or journals to the library from the following persons and organisations are gratefully acknowledged:

K.F. Pocock, Dr B.C. Rankine, Viticultural Publishing Inc.

Table 12. Email bulletins sent during 2002/2003

22/7/02	2002/2003 Agrochemical booklet availability and Dithane DF and Dithane M-45 information
26/7/02	New withholding period for Avatar and Legend
26/7/02	Selected addresses were used to send out the english translation of a negative article on Australian wines published in a Norwegian newspaper 20/7/02
29/7/02	Correction bulletin re information sent out on Thermovit in the 26/7/02 bulletin
5/8/02	AWRI wins the Maurice O'Shea Award 2002
15/8/02	Suggested wording for allergen labelling from 14 December 2002
30/8/02	Correction to page 6 of *Agrochemicals registered for use in Australian viticulture 2002/2003*
26/9/02	New registration of Shirlan and Crop Care Captan for phomopsis cane and leaf blight, and Mancozeb registration
26/9/02	Press release regarding the new GWRDC-funded project on allergies and wine
24/2/03	Management of mouldy fruit
11/3/03	Late season management of *Botrytis*

Links between viticultural and oenological research

Staff: Dr Sally-Jean Bell, Professor Peter Høj, Kevin Pardon

A meeting was conducted with approximately 18 industry representatives in May to review recommendations on the use of agrochemicals. Thereafter, 11,000 copies of the Institute's annual publication, *Agrochemicals registered for use in Australian viticulture 2002/2003* were produced and the information duplicated on the Institute's website. The recommendations were distributed to several wineries electronically to be reproduced in their own publications. The booklet was distributed with the *Australian & New Zealand Grapegrower & Winemaker*, *Technical Review* and in the *Research to Practice*™ IPM and Spray Application manuals. The tables were also featured in *Australian Viticulture*. The 2002/2003 MRLs for Australian export markets will be placed on the Institute's website (http://www.awri.com.au/agrochemicals/mrls/).

As a direct result of the Institute's agrochemical publication and it's Residue Analysis Service, Dr Sally Bell and Greg Ruediger devote a significant amount of time in liaising with major chemical companies. Companies are increasingly aware of the importance the wine industry places on meeting export market specifications. Thus they are keen to work more closely with the wine industry and the Institute to ensure that their products can be used in viticultural pest and disease programs in such a way that the maximum residue levels set by Australian export markets will not be exceeded.

During 2002/2003 Dr Sally Bell responded to 415 enquiries. The majority (68%) of enquiries were regarding the use of agrochemicals and the remaining 32% related to general viticulture.

Dr Sally Bell delivered a range of presentations at the Institute's Roadshows in Tasmania, Rutherglen (Vic), Bendigo (Vic), Pyrenees (Vic), Yarra Valley (Vic), Mornington Peninsula (Vic), Swan Valley (WA), Great Southern (WA) and Margaret River (WA) (see Appendix 1).

Dr Sally Bell participated in three *Research to Practice*™ Grapevine Nutrition workshops at Langhorne Creek (SA), Mildura (Vic) and Naracoorte (SA) and in two *Research to Practice*™ Winegrape Quality Management workshops at Great Southern (WA) and Swan Valley (WA) (see Appendix 1). She further prepared two articles entitled *Late season chemical control options for Botrytis* and *Why are recommended export withholding periods different to the withholding period stated on the product label?* for *Technical Review* and the September 2002 Murray Valley Winegrape Growers newsletter, respectively.

In the 2000/2001 season, a study commenced which aimed to investigate the effect of plant water status and canopy management on the phenolic composition of Shiraz grapes and wine. In the first season there were four treatments consisting of two canopy styles; Vertical Shoot Positioned (VSP) and Scott Henry (SH) and two levels of water status; high water (South end of rows) and low water (North end of rows). The differences in water status were due to differences in soil depth and a decrease in water pressure down the irrigation line (South to North). The difference in growing conditions resulted in measurable vigour differences. That is the vines at the North end of the rows exhibited low vigour while those at the South end exhibited high vigour. Results from 2000/2001 were reported in the 2002 Annual Report. In the second season of this trial (2001/2002) a change in treatments occurred. The pressure differential in the drip line was corrected and consequently no difference in plant water status could be detected. Therefore, water stress was absent in 2002 but the vigour difference was still apparent. These observations were confirmed by growth measures in the 2001/2002 season. The 2002 season was also markedly milder than 2001, as illustrated by the Lyndoch site Mean January Temperature (MJT). MJT in 2001 was 28.2˚C in comparison to the MJT in 2002 of 19.8˚C.

As seen in the previous season, the 2001/2002 low vigour vines produced higher concentrations of skin anthocyanin than high vigour vines, regardless of trellis type. Low vigour Scott Henry (SH) vines also produced higher seed catechin and epicatechin concentrations than those from high vigour SH vines. The smaller berry weight from the low vigour vines was a contributing factor to the higher concentrations observed. However, vigour had little effect on seed catechin and epicatechin concentrations in Vertical Shoot Positioned (VSP) vines. The difference in berry weight between high and low vigour VSP fruit was less than that of the high and low vigour SH fruit which may account for the lack of effect of vigour on VSP seed procyanidin concentrations.

High vigour VSP vines produced higher concentrations of seed catechin and epicatechin and skin anthocyanins than high vigour SH vines as seen in the previous season. However, in contrast it was the low vigour SH vines that produced higher concentrations of seed catechin and epicatechin and skin anthocyanins than low vigour VSP vines. As in the 2000/2001 season, the higher canopy density and lower bunch exposure exhibited by high vigour VSP vines in a mild season, appeared to be beneficial in terms of fruit phenolic concentration despite the bigger berries. In contrast to the previous season the low vigour SH vines with lower canopy density and higher bunch exposure and small berries increased fruit phenolic concentrations in comparison to low vigour VSP vines.

Sensory analysis of the 2001/2002 wines has been completed. The following is a brief summary prepared by Leigh Francis: Shiraz wines were made from grapes sourced from vines trained to either a Scott Henry or a Vertical Shoot Positioned trellis. In addition, a further treatment of high vigour versus low vigour was imposed. Paired comparison sensory difference tests for the attributes fruit aroma and overall astringency, were carried out. For the high vigour treatment no significant difference was found between the VSP and Scott Henry wines for either of the attributes tested. For the low vigour treatment, overall astringency was higher from the Scott Henry wine, but no difference in fruit aroma was found. While the grapes from the two low vigour treatments were picked at similar ripeness levels, the wines from the Scott Henry trellis treatment were higher in alcohol content than the VSP wines by approximately 0.7%, and the ripeness difference may account for the astringency difference observed.

These results have to be confirmed with data currently being acquired from the 2002/2003 season.

Preparation of information on wine and health issues

Staff: Creina Stockley, Peter Høj

Ms Creina Stockley, a clinical pharmacologist, has assumed the position of Health and Regulatory Information Manager since 1991, and has established, as part of her responsibilities, a database of research on the beneficial and detrimental health effects of alcohol and in particular, wine, on the internal database of the John Fornachon Memorial Library. This was facilitated by the subscription to relevant medical and scientific journals, and by the formal and informal exchange of information between complementary organisations, both national and international.

During the year, 96 independent information requests were received by the Health and Regulatory Information Manager on wine and health issues from industry, government and the general public. Subscription to relevant medical and other journals has continued. The journals have been regularly scanned, the database of research on the health effects of wine has been added to and articles have been prepared for inclusion in the Institute's publication, *Technical Review*, and for other Australian wine industry and international alcohol industry newsletters. Articles and other material have also been prepared for the electronic and print media. For example, three articles have been prepared for the bimonthly international publication, *AIM—Alcohol in moderation*, and one article for the quarterly newsletter of the Australian Society of Wine Education, in addition to two papers for the *Australian & New Zealand Grapegrower & Winemaker*. The Health and Regulatory Information Manager also co-authored with Professor Roland Stocker and Dr Ruth O'Halloran, formerly of the Heart Research Institute, a paper entitled *De-alcoholized red wine decreases lesion development in apolipoprotein E gene deficient mice* for the *American Journal of Clinical Nutrition* (accepted for publication); this paper ensues from the GWRDC-funded project HRI97/2 *Potential cardioprotective activities of wine components based on synergistic interaction with vitamin E*, which the Health and Regulatory Information Manager coordinated. A book chapter entitled *The potential therapeutic value of wine from a clinical and scientific perspective* has also been prepared for the book entitled *Handbook of enology: principles, practices and recent innovations*, edited by Dr V.K. Joshi, which is to be published by M/S Haworth Press Inc., New York. This book chapter reviews the extensive animal and human data generated since the publishing of the 'French Paradox' in 1992 on the potential biological mechanisms supporting the epidemiological evidence that the low to moderate consumption of wine reduces the risk of all-cause mortality. A book chapter on wine and health was also prepared for the book entitled *Australian wine styles and tastes* by Patrick Iland and Peter Gago.

Submissions prepared for the Australian wine industry include draft resolutions OENO/ASP/99/129/Step 7 *Health warning*, OENO/VINSAN/01/191/Step 7 *Specificity of wine and scientific research*, OENO/SCVNS/00/150/Step 6 *OIV/WHO relations* and OENO/ASP/01/190/Step 5 *Wine consumption and young adults* for the Nutrition and Health Subcommission, and a proposal on a preferred structure for the OIV Nutrition and Health Subcommission, which was presented at the 83rd 2003 General Assembly of the Office International de la Vigne et du Vin.

A booklet entitled *The A-Z of information on wine and health* was developed and published. The Commonwealth Department of Health and Aged Care favourably reviewed the booklet and contributed towards its publication and printing costs. As of 1 July 2003, approximately 48,500 booklets have been distributed to the general public, cellar door and other sales outlets in Australia, to the food and beverage industry, to health and allied health professionals, and to Lallemand in France. The booklet has also been favourably reviewed and publicised in the *Sydney Morning Herald* and in the *Cairnes Post.*

Project coordination

Through Creina Stockley, the Institute has played a coordinating and a participating role in three GWRDC-funded research projects on medical aspects of wine consumption entitled *Grape antioxidant phenolics: absorption and inhibition of lipid peroxidation in humans, Reduction of damage to LDL and DNA from oxidative free radicals by the regular and moderate consumption of wine*, and *Potential cardioprotective activities of wine components based on synergistic interaction with vitamin E*. All three projects have now been completed and the final reports can be viewed on http://www.gwrdc.com.au.

Workshop

A workshop was funded by the GWRDC to summarise the GWRDC-funded research outcomes for wine and health and to provide further direction for research in this area. The workshop was designed, developed and organised by Creina Stockley, and invited participants represented both industry and the research/scientific community. The workshop presentations and research outcomes have been documented in a final report, which also included potential new projects based on a prepared list of Australian research priorities for wine and health, as well as a list of criteria with which to evaluate and select future projects. The primary outcome of the GWRDC-funded research is that the wine- derived phenolic compounds are absorbed into the body and, of the beverages tested, the most efficacious to reduce and prevent the oxidation of low density lipoprotein (LDL), associated with atherosclerosis and cardiovascular disease, may be dealcoholised red wine. Background diet, disease and smoking status, however, appear to influence the antioxidant effects of the wine-derived phenolic compounds, which may act synergistically with the endogenous antioxidant, vitamin E. Workshop participants recommended further clinical research to confirm these results. Clinical research to determine whether moderate wine consumption is cardioprotective in two population groups at higher risk of cardiovascular disease - the obese and those with type 2 diabetes was also recommended by Workshop participants.

Technical and regulatory support to the Australian wine industry

Staff: Peter Høj, Creina Stockley

Information requests

One of the activities of the Institute has been to provide legal/regulatory and technical advice and assistance to the Australian wine industry, through the Health and Regulatory Information Manager, the Industry Services team and the Director. From 1 July 2002 until 30 June 2003, 206 independent information requests on scientific/technical and regulatory issues from the government and industry were fielded by the Health and Regulatory Information Manager (activities of the Industry Services team are reported elsewhere in this Report). The Director contributes advice of technical nature to a host of bodies and companies on an ongoing basis.

Industry Committee membership

During the year, additional support to the industry has been derived from the Director's membership on the AWBC International Trade Advisory Committee (on request) and the AWBC/WFA Wine Industry Technical Advisory Committee. One of the important aspects of the Institute's support of the Australian wine industry is its pivotal role in facilitating the triennial Australian Wine Industry Technical Conference (AWITC), in conjunction with the Australian Society of Viticulture and Oenology. Professor Peter Høj is the Chair of the 12th AWITC.

In further support of the industry, the Director served on the following committees and boards during the year: Wine Industry Technical Advisory Committee (AWBC/WFA); Board member of the Cooperative Research Centre for Viticulture; Board member of Provisor Pty Ltd; Management Committee for Viticultural Publishing Inc.; South Australian Premier's Wine Committee; Wine Committee of the Royal Agricultural and Horticultural Society of South Australia; Prime Minister's Science, Innovation and

Engineering Council; South Australian Premier's Science and Research Council.

The Health and Regulatory Information Manager was a member of the following committees: Wine Industry Technical Advisory Committee (as Technical Liaison); the AWBC Legislation Review Committee; the AWBC/WFA Wine Industry National Environment Committee; the Eco-efficiency Working Group (Sub-committee of SAWBIA's Environment Committee); and was Australian delegate for Office International de la Vigne et du Vin Nutrition and its Health Subcommission.

The Director and Health and Regulatory Information Manager also coordinate Course 3005WT *Grape industry practice, policy and communication* for the School of Agriculture and Wine at The University of Adelaide (Appendix 2). In its ninth year, 62 students enrolled in the Course, which exposes students to organizational, commercial, environmental, political and societal issues relating to the wine industry's operating environment.

Reviews and publications

During 2002/03, significant efforts, energies and resources have continued to be directed towards the labelling and production provisions for wine in the Food Standards Code of Australia and New Zealand, and further amendments entitled *Application to change Standard P4 of the [Australian] Food Standards Code and consequential amendments to Standard 4.1.1 of Volume 2 of the Food Standards Code of Australia and New Zealand* have been submitted to Food Standards Australia New Zealand in conjunction with the Winemakers' Federation of Australia. A guide *Winemaking provisions for wine in Australia and its export markets*, has been drafted and will be available on the Institute's website very early in the next financial year; this complements the Institute's other guide *Analytical specifications for export of Australian wine*, which was published in 1995 and 2001.

During 2002/2003, scientific and technical information and/or issues that have been reviewed, and material prepared includes: additives and processing aids used in Australian winemaking and their maximum limits for the Agriculture, Fisheries and Forestry Australia (AFFA) delegation to World Wine Producers' Group; regulatory and technical consequences of using mustard oil impregnated wax in winemaking; regulatory consequences of using milk as an agrochemical; regulatory and scientific consequences of spraying and washing soot-contaminated grapes with ethanol; natural versus synthetic tartaric acid; toxicological and technical issues related to the use of discrete pieces of oak versus barrels and staves; regulations relating to ethyl carbamate in grapes and wine; sources of bromide ions in wine; and the allergenic potential of processing aids used in winemaking. At the request of Government and industry, two papers were contributed to the 83rd 2003 General Assembly of the Office International de la Vigne et du Vin: Høj, P.B.; Stockley, C.S.; Pretorius, I.S. *The use of non-modified GMO-derived products in the development process of wine*; and Pretorius, I.S.; de Barros Lopes, M.A.; Høj, P.B. *Development of superior wine yeast: current status and future opportunities to meet the consumer challenge*. Professor Pretorius presented these papers at the OIV meeting in France in June 2003. Another paper on the contribution of the winemaking process to the concentration of lead in wine was published (Institute publication #715).

Technical Information is also disseminated to the Australian wine industry, including all grape and levy payers, by the Institute's bimonthly publication, *Technical Review*, of which the Health and Regulatory Information Manager was editor until 1 January 2003 (see further details elsewhere in this Report). This is in addition to the Institute's *Annual Report* edited by the Director and the Communication and Publicity Manager; the Institute's Roadshows in various winemaking regions (Appendix 1); through the remote web-accessible database of the John Fornachon Memorial Library (available only to Australian grapegrowers and winemakers); the Institute's website (www.awri.com.au); the Institute's Email Advice Bulletin on regulatory and technical issues; and through the many presentations given by Institute staff (Appendix 1).

This project of technical and regulatory support to the Australian wine industry is ongoing as technical and regulatory issues are regularly raised by industry or government, both in Australia and internationally. Furthermore, these issues often span several years.

The identification and measurement of potential allergens in wine

A collaborative project between the Department of Allergy, Asthma and Clinical Immunology, The Alfred Hospital and The Australian Wine Research Institute commenced on 1 July 2002. The project is funded by the GWRDC through The Alfred Hospital and Creina Stockley is the project supervisor and Professor Robyn O'Hehir is the principal investigator. The project was developed in response to labelling requirements for potential allergens introduced on 14 December 2002 by Food Standards Australia New Zealand, where use of the processing aids casein, egg white, isinglass and milk and milk products in winemaking requires declaration of use on the label of each wine and wine product.

The project comprises two sections. The first section is the determination of detectable allergenic proteins in wine, and the second section is a controlled laboratory trial of wine ingestion in subjects with confirmed allergy to eggs, fish, milk and nuts. Animal and human ethics approval has been received from the relevant ethics committees. One hundred commercially available wines have been collected, and sensitive and specific assays are currently being developed to identify and quantitate the concentration of processing aids in the wines. For example, a panel of relevant monoclonal antibodies (ovomucoid, casein, ß-lactoglobulin, and isinglass) is being generated for the development of enzyme immunoassays (ELISAs) to detect whether there are allergenic proteins in the wines; an ELISA has already been developed for ovalbumin using commercial polyclonal and monoclonal antibodies. Further evidence of detectable allergenic proteins in the wines will be

obtained from basophil activation tests (BAT) and flow cytometry, and challenge subjects with extreme sensitivity to the relevant food allergens are being recruited to provide a blood sample. BAT have been developed and optimised for use with wine and the processing aids. Analysis of the 100 wines using BAT is occurring in tandem with the clinical challenges of the second section of the project. Twenty-one of the 30 challenge subjects have been recruited and clinically characterised, and they will be compared to a group of 10 healthy subjects without a history of food or wine reactions and in the absence of specific IgE to any of the study allergens. It is anticipated that the project will be completed by 30 June 2004.

One manuscript outlining the scope and background of the project has been prepared and published in *The Australian & New Zealand Grapegrower & Winemaker* (Institute publication #705), and one television and five radio interviews have been conducted by Creina Stockley and Professor Robyn O'Hehir (Table 13), in conjunction with the publishing of two articles in the print media on allergen labelling for wine.

Quality Liaison Manager

Mark Gishen remains heavily involved in the collaborative research project evaluating the use of near infrared spectroscopy (NIRS) for the rapid determination of a number of compositional parameters in grapes, must, wine and grape spirit, and continues to take primary responsibility for the project as team leader. The details of this project are reported elsewhere in the Annual Report.

The major output of the Institute's activities in the provision of advice on quality management techniques to industry remains the *From grapes to glass* program, which was published in August 1997 and enhanced with a simple HACCP module in 1999. Industry interest remains greatest in the HACCP module - a simple program delivered in a one-day course that incorporates an HACCP-type (hazard analysis and critical control point) food safety plan. This module was designed to satisfy the requirements of the proposed (now delayed) changes to the food hygiene regulations, and meet the needs of the smaller scale businesses in the industry. Only one course for the HACCP module was conducted throughout the year, adding another six to the growing list of companies having attended (now totalling 94). The *From grapes to glass* program provides a simple and relatively cheap program that uses a staged approach in the attainment of internationally recognised standards, starting from the Codex HACCP principles and leading to the full ISO 9000 quality management standard.

Mark Gishen, the Institute's Quality Liaison Manager, takes primary responsibility for the internal quality management systems of the Analytical Service, overseeing management reviews, documentation, auditing, and corrective actions, and has now taken on the role of Authorised Representative in respect of its NATA accreditation. The quality system of the Analytical Service was reassessed by NATA against the ISO 17025 standard during December 2002 and the laboratory was granted continuing accreditation. Several changes that were made at the time of reassessment included the addition of Amanda Cook as a signatory for most of the chemical testing procedures, and Matt Holdstock's signatory status being extended to cover hydrometer calibrations. In addition, accreditation of methods was expanded to include two more methods being the determination of acetic acid in wine by enzymatic kit using the *Cobas* FARA, and the HPLC multi-residue method for agrochemicals. The Analytical Service continues to participate and excel in both national and international proficiency testing programs for routine wine analysis and for agrochemical residue testing. The Analytical Service continues to manage its quality management system with the aid of the *Paradigm Quality* software package. The software is gradually being introduced throughout the Institute in general, primarily as a means of assisting control and availability of policy and procedural documentation, as part of a general move toward quality management systems implementation.

Mark Gishen is the Institute's representative on the Winemakers' Federation of Australia (WFA) and Winegrape Growers' Council of Australia's (WGCA) recently formed working group known as the Legal Metrology Group. This group was formed in response to a proposal put by the National Standards Commission and aims to develop a metrological control system for measurement instruments used by wineries for the payment for winegrapes. The group has prioritised the various areas to be addressed and has drafted a work program to progress its aims.

Communication and Publicity Manager

The Communication and Publicity Manager's role is a part-time position for Rae Blair, who is also the Personal Assistant to the Director and the Conference Manager of the Australian Wine Industry Technical Conference (details of her other activities can be found under the report for *Provision of Technical Information)*. As Communication and Publicity Manager, she is responsible for ensuring that industry and stakeholder groups receive a clear understanding of the positioning (value) of the Institute. This positioning is developed in line with the Institute's mission statement and business plan objectives. Part of her role is to coordinate the Institute's printed material and other non-technical communications, and to act as a conduit for media. The Communication and Publicity Manager is also responsible for the performance and output of the John Fornachon Memorial Library. The report of the activities of the Library can be found elsewhere in this Report.

Media contacts and interviews

Institute staff were interviewed by representatives from various forms of media over the year (Table 13).

The following press releases were prepared and distributed:

> Appointment of the Director of Research, Professor Sakkie Pretorius

> Appointment of Peter Wall as Chairman, and Dr Darren Oemcke as Chief Executive Officer of Provisor.

General enquiries* responded to by the Communication and Publicity Manager during the year:

Students	11
Industry	7
Consultants	1
Government	1
Media	2
Other	19
Total	51

*With the Institute's website being more widely known and used, the Communication Manager receives regular requests for information (as her email address is listed as the 'general enquiries' contact address). Many requests for information are simply forwarded to the relevant expert, however, there are many that can be dealt with without forwarding. These figures only represent those enquiries that were responded to by the Communication Manager and do not represent the total enquiries received.

Table 13. Media interviews of Institute staff for 2002/2003

Date	Staff member	Item discussed	Media details
8/7/02	Peter Godden	36 month results from the closure trial	> UK freelance journalist, Jamie Goode, Wineanorak.com, *Harpers*
11/7/02	Peter Godden	GMOs and the wine industry	> Max Allen, *The Weekend Australian*
12/7/02	Peter Godden	Cork issues with emphasis on closure trial	> Susie Barrie, *Decanter*
15/7/02	Mark Gishen	NIR project	> Michael Sexton, *7:30 Report* (ABC TV)
17/7/02	Peter Godden	Changes that occur in wine when decanted and aerated, and differences in sensory perception attributable to the shape of the glass	> In studio recorded interview with Alan Saunders, host of *The Comfort Zone* ABC radio (aired 20/7/02)
29/7/02 30/7/02	Peter Godden	Closure trial	> Emailed: Michael James, ABCNEWS.com, USA > Live-to-air interview with Kevin Norton, ABC Radio, Adelaide *Drive*
8/8/02	Peter Godden	Incidence of TCA in wine sold in Australia with reference to statistics from the *Advanced Wine Assessment Course*	> Tim White, *Australian Financial Review* (published 10 and 17 August)
14/8/02	Peter Godden	Results of closure trial and closure issues	> Robyn Smith, ABC TV News and current affairs
15/8/02	Peter Godden	The role of the AWRI, with reference to Roadshows, and the closure trial	> Bruce Mounster, *Tasmanian Country* newspaper (published 23 August 2002)
19/8/02	Peter Godden	Issues relating to *Brettanomyces* and wine	> Philip Rich, *Australian Financial Review*
21/8/02	Peter Godden	Closures and bottle ageing of wine	> UK freelance journalist, Sally Easton, *Drinks Business*
23/8/03	Peter Godden	Discussion of the 36 month results from the closure trial	> Jo Burzynska, Crier Media Group UK, Editor of *Drinks Buyer Europe*
23/8/02	Peter Godden	AWRI/Roadshows	> Bruce Mounster, *Tasmanian Country* newspaper
26/8/02	Peter Høj	GMOs in the wine industry	> Ben Canaider (*Age Epicure*)
29/8/02	Peter Godden	AWRI/Roadshows	> Win Television (Channel 9) on 6 pm News throughout Victoria except greater Melbourne and Geelong > ABC Radio Drive, broadcast on 29th August throughout Victoria except central Melbourne > *The Bendigo Times*, story was to be run on Friday 30th August
4/9/02	Peter Godden	Further information on article on changes that occur in bottled wine	> UK freelance journalist, Sally Easton, *Drinks Business* magazine
5/9/02	Peter Høj and Creina Stockley	Allergens in wine	> Spoke with Max Allen
12/9/02 13/9/02	Peter Godden	AWRI/Roadshow	> David King, *The Press* - the Christchurch/South Island newspaper > Ceinwen Parish, Radio New Zealand
19/9/02	Peter Godden	Information for article on closures and discussion of closure trial	> Nicki Bourlioufas, *Liquor Watch* magazine, UK
24/9/02 25/9/02 26/9/02	Creina Stockley	GWRDC-funded allergen study and wine industry position on allergen warning labels.	> Robert McClean, arranged by Belinda Byrne of *Channel 9 News* (screened 25/9/02) > Liam Bartlett, ABC Radio Western Australia and Perth > David Anderson, ABC Radio Queensland and ABC Capricornia > Derryn Hinch, 3AK (Melbourne) > Susie Hamilton, ABC Radio Renmark > This is in relation to the two articles in *The Weekend Australian* (14 and 15/9/02) and *The Australian* (25/9/02).
25/9/02	Peter Godden	Cork and TCA, with particular reference to UK Wine and Spirit Association study	> UK freelance journalist, Jamie Goode, Wineanorak.com, published in *Harpers* and referred to in *Decanter* magazine, December 2002
27/9/02	Creina Stockley	Health benefits of seed/skin enriched wine	> Dan Nolan, *Channel 10 News* (Brisbane)
31/10/02	Peter Godden	Discussion of research findings (USA) that glass shape has an effect on wine flavour	> Jeremy Roberts, *The Australian*
31/10/02	Peter Godden	Allergen wine labelling – origins of and reasons for using certain wine additives and processing aids	> Jessica Lawrence, *Sunday Mail*, Brisbane
1/11/02	Peter Godden	Discussion of 36 month results from closure trial	> Birgitt Baader, *Choice*
1/11/02	Creina Stockley	Allergen labelling	> Provided information to Lynn Alley, Wine Spectator
4/11/02	Peter Godden	Re Jamie Goodes' article in *Harpers* and quotes attributed to PWG.	> David Bird, Freelance journalist from UK
8/11/02	Peter Godden	Screw cap usage in Australia and NZ and closure issues in general	> Alexandra Rundel, Editor for *Saveur* magazine (New York, USA)
8/11/02	Miguel de Barros Lopes	Genetic modification	> Graham Lawton, *New Scientist,* United Kingdom
15/11/02	Peter Godden	*Brettanomyces* and wine	> Tim Atkins, *Guardian/Sunday Observer*, UK
28/11/02	Miguel de Barros Lopes	Biotechnology in winemaking	> Graham O'Neil, Australian *Biotechnology News*
28/11/02	Sally Bell	Grape crops and salinity	> Guy Webber, *The Stock Journal*
2/12/02	Peter Godden	Attempts to develop various methods for the removal of TCA from corks	> Max Allen, *Weekend Australian*
9/12/02	Peter Godden	Screw caps and the closure trial	> Bernard Carpinter, *The Dominion Post,* Wellington NZ
23/12/02	Peter Godden	Closure permeation – reference to wine development and 'reductive' aromas	> Jo Burzynska, Crier Media Group, UK, Editor of *Drinks Buyer Europe*
30/12/02	Peter Godden	Ageing of red wine under screw cap	> Tyson Stelzer, www.cellaringwine.com
30/12/02	Peter Godden	Quality factors for sparking wine/Champagne	> Alexia Moses, *Sydney Morning Herald*
20/1/03	Peter Høj	Future role of R&D for wine industry success	> Jeremy Oliver

Date	Staff member	Item discussed	Media details
30/1/03	Creina Stockley	Past and future directions and outcomes of research into health benefits of red wine	> Tamara Hunter, *The West Australian*
6-8/2/03	Peter Høj	Various issues regarding age and yield of Australian vineyards	> Jancis Robinson
3/1/03	Peter Godden	Wine labeling with regard to allergens / origins of and reasons for using certain wine additives and processing aids.	> Jessica Lawrence, *Sunday Mail*, Brisbane. Also article in *Sunday Mail*, Adelaide, 5 January 2003.
13/1/03	Peter Godden	Effect of increased alcohol on the perception of flavour and aroma in wine.	> Tim White, *Australian Financial Review*
17/1/03	Peter Godden	Effect of drought and very high temperatures on the potential quality of 2003 harvest	> Andrew McGarry, *The Weekend Australian* (Information included in an article published on 18 January 2003)
7/2/03	Peter Godden	Comment on *Choice* magazine survey on consumer acceptance of screw caps for red and white wines	> Kim Arlington, Associated Press
20/2/03	Peter Godden	Possible effect of rain on the wine industry and vintage	> Jessica Rich, *Channel 9 Network News* (aired 20 February 2003)
27/2/03	Peter Godden	Closure issues, with particular reference to synthetic closure use in Australia, and for Australian wine sold in the UK.	> Mark Chipperfield, *Sunday Telegraph*, London
28/2/03	Alan Pollnitz	Oak	> *Sydney Morning Herald*
5/3/03	Peter Godden	Information for article on Piedmontese, particularly Barolo, wines.	> Tim White, *Australian Financial Review* (Full-page story published 8 March).
7/3/03	Peter Godden	*Brettanomyces*	> Jamie Goode, article for *Harpers* magazine
17/3/03 18/3/03	Peter Godden	Effects of bushfire smoke on grapes and wine	> Katie Fisher, Weekly Times, Melbourne > Huon Hooke *Sydney Morning Herald* and *Melbourne Age* (articles published 1 April 2003). Article also discussed on *ABC News Radio* on 1 April 2003.
19/3/03	Peter Godden	General perception of vintage, particularly bushfire smoke issue	> Max Allen, *Harpers Magazine*
20/3/03	Peter Godden	Information on closure trial	> Tina Vierra, *Practical Winery & Vineyard USA*
26/3/03	Peter Godden	Percentage of TCA tainted wines in Australia	> Kirsty Wilcox, *Uncorked Magazine* (*Sydney Morning Herald* and *Melbourne Age*)
1/4/03	Peter Godden	Bushfire smoke taint in grapes/wines	> Louise Marr, ABC *Radio 666 Canberra*, live to air interview
2/4/03			> Chris Snow, *Drinks Bulletin* magazine, UK
4/4/03	Peter Godden	Technologies used in the production of grapes and wine.	> Kristi Essick, *The Wall Street Journal*, European bureau
8/4/03	Peter Godden	The use of supercritical CO2 to remove TCA from cork, and other issues regarding the technical requirements of wine bottle closures in general.	> Giselle Weiss, *The Economist*, European bureau
28/4/03	Peter Godden	Methods of removing TCA from cork	> Jamie Goode, *Harpers* magazine, UK.
28/4/03	Peter Godden	*Brettanomyces*	> Jenny Pollack, *Bent* magazine
9/5/03	Peter Godden	Closure trail, and cork/closure issues	> Cyril Penn, *Wine Business Monthly*, magazine and web site, USA
20/5/03 26/5/03	Peter Godden	Closure issues	> Tejinder Gill, *The Australian and New Zealand Wine Industry Journal* > Kylie Stevenson, *Liquorwatch*
26/5/03	Peter Godden	Objective and subjective measurement/definition of wine 'quality'.	> Tim White, *Australian Financial Review* (Full-page story published 31 May)
2/6/03	Peter Godden	Sources of TCA in wine and statistics on incidence of TCA taint in wine.	> Joy Walterfang, *Winestate* magazine
4/6/03	Peter Godden	*Brettanomyces* issues, particularly control strategy	> James Kelly, *The Australian & New Zealand Grapegrower & Winemaker*
5/6/03	Peter Godden	Reverse osmosis method to remove 'bushfire smoke taint' from wine	> Katie Fisher, *Weekly Times* newspaper, Melbourne > Liz Rodway, *ABC Radio*, Shepparton Victoria
6/6/03	Peter Godden	The use of protein analysis for varietal identification of juice and wine	> Max Allen, *The Weekend Australian*
12/6/03	Peter Godden	Issues relating to the effects of bushfire smoke on grapes and wine. Debate about the use of oak chips versus barrels. Closure issues, particularly screw cap industry and consumer acceptance	> Kathy Bolt, *Australian Financial Review*
16/6/03 18/6/03	Peter Godden	The use of protein analysis for varietal identification of juice and wine	> Melody Horrill, *Channel 10 News*, Adelaide > Tony Love, *The Advertiser*, Adelaide
19/6/03	Peter Godden	Issues relating to *Dekkera/Brettanomyces* and wine, and sensory thresholds for four-ethylphenol in red wines	> Tim White, *Australian Financial Review*
6/6/03	Peter Høj	Australian wine industry	> Willy Billiard, *Vigne et Vin* International Publications

Staff: Don Buick, Matthew Holdstock, Greg Ruediger, Amanda Cook, Matthew Cream, Randell Taylor, Sandra Lloyd-Davies, David Boehm, Athina Massis, Anna Catalano, Danielle Leedham, Maria Mills, Heather Brooks and Belinda Bramley. Peter Eichinger commenced in June 2003.

L to R Back row: Athina Massis, Maria Mills, David Boehm, Randell Taylor, Matthew Cream, Matthew Holdstock, Anna Catalano
Middle row: Greg Ruediger, Sandra Lloyd-Davies, Amanda Cook, Heather Brooks, Danielle Leedham, Jelena Jovanovic
Front row: Peter Eichinger, Belinda Bramley

The Analytical Service (AS) is divided into three sections namely the analytical laboratory supervised by Matt Holdstock, the trace analysis laboratory supervised by Greg Ruediger and the administration section supervised by Sandra Lloyd-Davies.

The AS provides a commercial testing facility operating independently within the Institute. It is self-funding in all respects from its fee earning work, and any surplus generated is returned to the Institute for the benefit of the Australian wine industry. It is focussed solely on providing services to the Australian wine industry, with a commitment to providing many specialised services that are not generally available in winery laboratories, or from other commercial laboratories. AS interacts with, and provides analyses for other groups within the Institute (Industry Services and Research groups) on a fee for service basis, although this constitutes a minor part of the AS activities.

Our aim is to provide a range of quality analytical services that are important to industry which can be provided in a reliable, price competitive, accurate and timely manner. During December 2002, a market research survey was commissioned (McGregor Tan Research) in which about 200 wineries and suppliers to the industry were questioned on their knowledge of the AS's services and performance. The results of the survey were very positive, with a very high (96%) awareness of the laboratory's existence and the range of services offered. The clients identified their key requirements being accuracy, reliability, strong technical expertise, NATA accreditation and timeliness, as well as good customer service, confidentiality and reputation. The AS was rated very highly on all of these aspects of its performance. A new fee schedule was prepared and mailed out to levy payers early in the financial year.

Overall, the Analytical Service conducted approximately 54,000 individual tests on wine and grapes in 2002/2003. These services included export certification and support for the quality control activities of winemaking and viticulture. The growing interest in grape quality has led to increasing numbers of grape samples being tested for colour, glycosyl-glucose, brix, pH and titratable acidity over the last two vintages. While many large wineries do have testing facilities, we receive samples from growers and consultants to independently test grapes as this often impacts on grower payments.

The number of tests performed by the Analytical Service increased by 12% from the previous year. Significant increases occurred in the number of AWBC certificates and EU certificates, but the number of Japanese certificates has declined due to sluggish economic conditions there. It should be noted that some of the major exporters have developed NATA accredited laboratories and are thus able to issue their own analytical certificates. Testing for chloroanisoles and 4-ethylphenol increased with the latter being related to growing concern with *Brettanomyces* spoilage of wine and the associated Industry Services investigation. The number of data points produced increased by 29% indicating many of the increased testing numbers were for multiple analyte testing. The performance of the laboratory in a range of analytical classes is given in Table 14.

Table 14: Comparison of selected tests performed by period

	98/99	99/00	00/01	01/02	02/03
Total number of tests	39,087	44,846	46,037	48,314	53,932

There have been some significant staffing changes in the last year. Most notable has been the retirement of Don Buick, the Manager of Analytical Services. He has overseen considerable growth in the business, provided sound engaging leadership and overseen a number of reforms in the laboratory to improve efficiency and quality. Dr Peter Eichinger has taken over this role, having experience in analytical chemistry and chemical research and will be endeavouring to continue this focus. After long and dedicated service, John Hughes left Analytical Services in September 2002, and his replacement, Sandra Lloyd-Davies, has proven to be very capable and adaptable. In light of these departures and staff development, the signatory status of Matthew Cream has been upgraded and Peter Eichinger has attained accreditation for all tests.

Matt Holdstock, Analytical Service Supervisor, has completed his studies towards a Graduate Diploma in Oenology. The Service, therefore, now has two qualified oenologists to help enhance the service and advice that can be offered to industry. Matt's completion of his studies will allow him to provide greater depth in dealing with client enquiries related to the services we provide. Matt has also been involved with the Inter-Winery Analysis Group (IWAG). The purpose of this group is to expedite inter-laboratory proficiency trials and enable a comparison of results obtained in different laboratories using a range of techniques.

Improvements in quality systems are seen as being very important. To aid in tracking document versions, we use a software program known as *Paradigm II* (further details are provided under the Quality Liaison Manager's report). A number of other functions are proving valuable, as the alerting facility has been set up to automate tasks such as scheduling ongoing calibrations of instruments, method reviews, audits, etc.

An advisory visit from NATA to provide information about implementation of the OECD GLP guidelines to residues testing for agrochemicals used in the wine industry occurred in September 2002. Since that time, significant progress has been made to achieve compliance, and an audited trial under these guidelines is still to be assessed before an accreditation visit occurs. This has been delayed in part due to Long Service Leave taken by Greg Ruediger. The National Residue Association[2] (NRA) requirements for registration of agrochemicals under OECD GLP testing conditions has been delayed, and therefore has not impacted upon the Institute.

This year AS purchased a second GC/MS, this time with headspace SPME capabilities, which arrived in the third quarter of the financial year. In the short term, the GC/MS capabilities will be extremely useful to reduce turn-round times and the current backlog of work. Once this occurs, it is envisaged that we will be able to undertake some method development utilising the SPME capabilities. The addition of the automated headspace capabilities will complement existing services in the new year. Several staff have attended specialised instrument training courses to ensure new equipment is well maintained and used to its maximum capability.

With the assistance of FOSS Pacific, a FOSS Winescan 2000 was installed late 2002. The full capabilities of this instrument have yet to be confirmed. While calibrations exist for European wines, these are unlikely to be optimal for Australian wines. Calibrations for Australian wine samples are proceeding very well, with a large number of dry white and red wines represented in the calibration curves. Sweet white wines are poorly represented in the calibrations, and are needed. We wish to expand the calibrations in this area to ensure these wines will be covered. Matt Holdstock attended a FOSS meeting in New Zealand and discussed the ongoing calibration and other experiences from Winescan users.

A Miele dishwasher (for laboratory glassware) was installed in September 2002, and this has proven to be a great time and labour saving device.

As previously mentioned, Greg Ruediger has taken most of his Long Service Leave during the April quarter. His role as Supervisor of the trace analytical laboratory (TAL) was very ably handled by Randell Taylor. Belinda Bramley was recruited as a Technican in that laboratory to assist with the extra workload and has settled in quickly. Heather Brooks has developed quickly and has attended training courses on the Agilent HPLC operating software (Chemstation). Many aspects of this software is common to the Agilent GC/MS platform and so we expect she will gradually be able to run sequences on this instrument too.

The trace analysis laboratory has been involved in a number of residue trials involving the use of agrochemicals. This has involved both testing of residue levels for registration, as well as small-scale winemaking trials to evaluate the impact of these compounds on fermentation and organoleptic qualities. This work will continue for several months into the new financial year.

The staff in the Analytical Service group frequently interact strongly with the staff members of Industry Services. As outlined earlier in this report, an example of this is the Institute's response to the smoke-affected grapes from the bushfires in North Eastern Victoria. While direct fire damage to the growers was averted, smoke exposure had a profound affect. A large number of samples (over 200) were tested for guaiacol and 4-methylguaiacol as indicators of the extent of smoke damage, as well as the effectiveness of various treatments to reduce smoke taint (see full report under *Investigations conducted into the nature and amelioration of taints in grapes and wine, caused by smoke resulting from bushfires*).

Staff have worked closely with the Industry Services team to analyse samples from a research closure trial at the 48 month stage (see report under *Evaluation of new analytical techniques and of processing aids for winemaking*), as well as initial and six month testing of a commercial closure trial. The analysis of large numbers of free and total sulfur dioxide and OD_{420} were performed with staff working in shifts to complete the work within two to three days of commencement. The commercial trial will involve client-submitted closures, reference corks and screw caps, as well as a small research component.

In continuing its support of improving the quality of Australian wine, the AS sponsored a trophy for the best Riesling at the Adelaide Wine show in September 2002.

[2]The NRA has recently undergone a name change to the Australian Pesticide and Veterinary Medicine Authority (APVMA), which is due to be gazetted shortly.

STATEMENT OF FINANCIAL PERFORMANCE
FOR THE YEAR ENDED 30 JUNE 2003

	2003 $	2002 $
Revenue from operating activities		
Grape and Wine Research and Development Corporation		
Project funds	4,324,916	3,849,613
Capital grants		
Building	0	0
Equipment	438,420	364,200
CRCV project funds	580,282	474,403
Commercial research collaborations	96,170	77,548
Analytical Service	1,507,854	1,224,875
Assistance by SA Government to Provisor Pty Ltd	700,000	0
Other revenue	340,644	281,279
Expenses from operating activities		
Employee benefit expense	4,281,915	3,876,256
Analytical and project operating expenses	1,194,694	1,044,055
SA Government assistance to Provisor Pty Ltd	700,000	0
Administration and general service expenses	557,898	518,789
Depreciation and amortisation expenses	471,029	439,572
Travel expenses	161,791	109,085
Borrowing cost expense	0	2,112
Profit from operating activities	620,959	282,049
Net gain (loss) on disposal of assets		
Motor vehicles	0	14,503
Equipment	(12,720)	(5,323)
Profit from ordinary activities	**608,239**	**291,229**
Total changes in equity	**608,239**	**291,229**

STATEMENT OF FINANCIAL POSITION
AS AT 30 JUNE 2003

	2003 $	2002 $
Current assets		
Cash assets	1,416,954	1,428,325
Receivables	511,693	300,761
Commercial bills	1,253,916	1,192,000
Other current assets	68,745	64,729
Total current assets	3,251,308	2,985,815
Non current assets		
Leasehold buildings	1,522,048	1,559,247
Plant and equipment	2,070,026	1,834,661
Investment In Provisor Pty Ltd	650,000	0
Australian Wine Industry Chair of Oenology	840,000	840,000
Total non current assets	5,082,074	4,233,908
TOTAL ASSETS	8,333,382	7,219,723
Current liabilities		
Payables and accruals	1,482,678	1,352,485
Project funds not expended and repayable		
GWRDC	191,589	208,055
CRCV	17,345	22,340
Unpaid investment in Provisor Pty Ltd	323,186	0
Provisions	624,457	575,068
Total current liabilities	2,639,255	2,157,948
Non current liabilities		
Provisions	67,411	43,298
Total non current liabilities	67,411	43,298
TOTAL LIABILITIES	2,706,666	2,201,246
NET ASSETS	5,626,716	5,018,477
EQUITY		
Reserves	966,750	966,750
Retained profits	4,659,966	4,051,727
TOTAL EQUITY	5,626,716	5,018,477

STATEMENT OF CASH FLOWS
FOR THE YEAR ENDED 30 JUNE 2003

	2003 $	2002 $
CASH FLOWS FROM OPERATING ACTIVITIES		
Grants and other income	7,665,762	6,196,141
Interest received	112,541	57,727
Payments to suppliers and employees	(6,719,029)	(4,794,415)
Net cash provided by operating activities	1,059,274	1,459,453
CASH FLOWS FROM INVESTING ACTIVITIES		
Receipt from SA Government for Provisor Pty Ltd	700,000	0
Payments for commercial bills	(61,916)	(522,000)
Payment for investment in Provisor Pty Ltd	(326,814)	0
Payments for plant and equipment	(682,215)	(347,017)
SA Government Assistance paid to Provisor Pty Ltd	(700,000)	0
Proceeds from sale of plant and equipment	300	37,091
Net cash used in investing activities	(1,070,645)	(831,926)
CASH FLOWS FROM FINANCING ACTIVITIES		
Repayment of loans	0	(120,000)
Net cash provided by financing activities	0	(120,000)
Net increase (decrease) in cash held	(11,371)	507,527
Cash at 1 July	1,428,325	920,798
Cash at 30 June	1,416,954	1,428,325
Reconciliation of net cash provided by ordinary activities with profit		
Profit from ordinary activities	608,239	291,229
Non cash flows in operating profit		
Amortisation and depreciation	471,029	439,572
(Profit) loss on the sale of plant and equipment	12,720	(9,180)
Charges to (reduction in) provisions	73,502	135,906
Changes in assets and liabilities		
(Increase) decrease in receivables and prepayments	(214,948)	(5,580)
Increase (decrease) in payables and accruals and project funds unexpended	108,732	607,506
Net cash provided by ordinary activities	1,059,274	1,459,453

APPENDIX 1. External seminars, talks and poster papers presented by Institute staff during 2002/03

Staff	Title of talk	Where and to whom	Date
P.A. Henschke, J.M. Eglinton, P.J. Costello, I.L. Francis, H. Gockowiak, A. Soden, P.B. Høj	Winemaking with selected strains of non-*Saccharomyces cerevisiae* yeasts and their influence on Chardonnay wine composition and flavour	13th International Oenology Symposium, Montpellier, France	11 Jun 02 (not previously reported)
P.A. Henschke and E.J. Bartowsky	Yeast interaction with grape phenolics and effect on wine sensory properties	Lallemand visit, Urrbrae, SA	25 Jun 02
P.A. Henschke P.J. Costello, P.R. Grbin[1]	Causes and control of mousiness in wine	Roadshow seminar to: Coonawarra, SA	2 Jul 02
I.L. Francis	Tannins and mouth-feel: terminology and methods for sensory evaluation of red wines		
M. Gishen	The influence of deficit irrigation on salt accumulation in grapes, and wine quality		
A.P. Pollnitz	Measuring desirable oak wood components in wine		
M. Gishen	The use of NIR for the measurement of grape, juice and wine components		
I.L. Francis	Oxidation of bottled wine: the role of ascorbic acid, and bottle storage conditions/non destructive measurement of white wine colour		
P.W. Godden	*Dekkera/Brettanomyces*, and the production of 4-ethylphenol during winemaking		
J.M. Eglinton and P.A. Henschke	Winemaking with alternative yeasts: influence on wine composition and flavour		
A.P. Pollnitz	Cork taint – causes, detection and prevention		
P.W. Godden	Update on the AWRI trial of the technical performance of wine closures		
E.J. Bartowsky and P.A. Henschke	Flavour aspects of MLF – control of the 'buttery' diacetyl character in wine		
M.J. Herderich	Polyphenols, 'pigmented polymers' and red wine colour: results from the Tannin Project, including the 2001 large-scale winemaking trial		
M.A. de Barros Lopes	Ascertaining the degree of genetic variation between strains of *Dekkera/Brettanomyces* yeast isolated from different Australian wineries and regions	ASVO Viticulture and Oenology Seminars, Tanunda, SA	10-11 Jul 02
P.W. Godden	Soil type and climatic influences on the wines of Bordeaux Geographic, viticultural and winemaking factors which influence the wines of Sauternes and Barsac	*Working With Wine Seminar - Bordeaux.* Negociants Australia (S. Smith and Son)	18 Jul 02
P.B. Høj	From molecular research to enjoyment of wine – the need for an integrated approach	*CSIRO Forum: the science and art of wine quality,* Urrbrae, SA	26 Jul 02
R.E. Day[2]	The art and science of wine quality		
K.S. Howell, E.J. Bartowsky, G.H. Fleet[3], P.A. Henschke	Yeast strain dynamics during mixed culture wine fermentation and effect on wine composition	International Union of Microbiological Societies World Congresses on 'The World of Microbes', Paris, France	27 Jul 02
P.B. Høj	McWilliam's Maurice O'Shea Award 2002 acceptance speech	McWilliam's Maurice O'Shea Award 2002 dinner, Sydney, NSW	2 Aug 02
R.G. Dambergs	Applications of NIRS in the wine industry	CSIRO Plant Industry	9 Aug 02
E.J. Waters	Oxidation of bottled wine: the role of ascorbic acid, and bottle storage conditions/non destructive measurement of white wine colour	Roadshow seminar: Hobart, Tas	15 Aug 02
J.M. Eglinton and P.A. Henschke	The role of VA in restarting stuck fermentation		
P.W. Godden	Managing stuck fermentation		
M. Gishen	Quality management systems – ISO 9000, HACCP – what are they, do you need them?		
S-J. Bell	Agrochemical issues for grapegrowers and winemakers		
J.M. Eglinton and P.A. Henschke	Winemaking with alternative yeasts: influence on wine composition and flavour		
M.J. Herderich	Polyphenols, 'pigmented polymers' and red wine colour: results from the Tannin Project, including the 2001 large-scale winemaking trial		
P.W. Godden	*Dekkera/Brettanomyces*, and the production of 4-ethylphenol during winemaking		
E.J. Bartowsky, D. Xia[3], G.H. Fleet[3], P.A. Henschke (presented by J.M. Eglinton)	Microbial induced oxidative spoilage of bottled red wine		
M. Gishen	The use of NIR for the measurement of grape, juice and wine components		
E.J. Waters	Tannins and mouth-feel: terminology and methods for sensory evaluation of red wines		
R.G. Dambergs	NIRS in the wine industry	Wirra Wirra Growers' Day	27 Aug 02

Staff	Title of talk	Where and to whom	Date
P.A. Henschke, P.J. Costello, P.R. Grbin[1]	Causes and control of mousiness in wine	Roadshow seminar to: Rutherglen, Vic	27 Aug 02
P.W. Godden	Managing stuck fermentation		
E.J. Waters	The link between vineyard and winemaking practices and wine protein stability		
P.J. Costello	Strategies for successful induction of malolactic fermentation		
P.W. Godden	*Dekkera/Brettanomyces*, and the production of 4-ethylphenol during winemaking		
S-J. Bell	Meeting salt specifications in winemaking		
E.J. Bartowsky, D. Xia[3], G.H. Fleet[3], P.A. Henschke (presented by P.J. Costello)	Microbial induced oxidative spoilage of bottled red wine		
J.M. Eglinton and P.A. Henschke	Winemaking with alternative yeasts: influence on wine composition and flavour		
E.J. Waters	Oxidation of bottled wine: the role of ascorbic acid, and bottle storage conditions/non-destructive measurement of white wine colour		
P.J. Costello, E.J. Bartowsky, P.A. Henschke	Which bacterial strains and how many are conducting your MLF		
P.A. Henschke	The role of VA in re-starting stuck fermentation		
M.J. Herderich	Polyphenols, 'pigmented polymers' and red wine colour: results from the Tannin Project, including the 2001 large-scale winemaking trial		
P.A. Henschke, P.J. Costello, P.R. Grbin[1]	Causes and control of mousiness in wine	Roadshow seminar to: Bendigo, Vic	29 Aug 02
S-J. Bell	Managing *Botrytis* in the vineyard		
P.W. Godden	Update on the AWRI trial of the technical performance of wine closures		
E.J. Waters	Reducing bentonite requirements for protein stability of wine by using heat and enzymes		
P.W. Godden	*Dekkera/Brettanomyces*, and the production of 4-ethylphenol during winemaking		
E.J. Bartowsky, D. Xia[3], G.H. Fleet[3], P.A. Henschke (presented by P.J. Costello)	Microbial induced oxidative spoilage of bottled red wine		
E.J. Waters	Oxidation of bottled wine: the role of ascorbic acid, and bottle storage conditions/non-destructive measurement of white wine colour		
J.M. Eglinton and P.A. Henschke	Winemaking with alternative yeasts: influence on wine composition and flavour		
M.J. Herderich	Polyphenols, pigmented polymers and red wine colour: results from the Tannin Project, including the 2001 large-scale winemaking trial		
P.A. Henschke, P.J. Costello, P.R. Grbin[1]	Causes and control of mousiness in wine	Roadshow seminar to: Pyrenees, Vic	30 Aug 02
S-J. Bell	Managing *Botrytis* in the vineyard		
P.W. Godden	Update on the AWRI trial of the technical performance of wine closures		
E.J. Waters	Reducing bentonite requirements for protein stability of wine by using heat and enzymes		
P.W. Godden	*Dekkera/Brettanomyces*, and the production of 4-ethylphenol during winemaking		
E.J. Bartowsky, D. Xia[3], G.H. Fleet[3], P.A. Henschke (presented by P.J. Costello)	Microbial induced oxidative spoilage of bottled red wine		
E.J. Waters	Oxidation of bottled wine: the role of ascorbic acid, and bottle storage conditions/non-destructive measurement of white wine colour		
J.M. Eglinton and P.A. Henschke	Winemaking with alternative yeasts: influence on wine composition and flavour		
M.J. Herderich	Polyphenols, 'pigmented polymers' and red wine colour: results from the Tannin Project, including the 2001 large-scale winemaking trial		
S. Vidal	Mouth-feel properties of proanthocyanidins: influence of proanthocyanidin structure and of interactions with wine components	XXI International Conference on Polyphenols, Morocco	9-12 Sept 02
P.W. Godden, I.L. Francis, J.B. Field[4], M. Gishen, A.D. Coulter, P.J. Valente, K. Lattey, P.B. Høj, E.M.C. Robinson	Closures - results from the AWRI trial three years post bottling	The Romeo Bragato Conference, Christchurch, New Zealand	12-14 Sept 02

Staff	Title of talk	Where and to whom	Date
A.P. Pollnitz	Cork taint – causes, detection and prevention	Roadshow seminar:	17 Sept 02
P.W. Godden	Managing stuck fermentation	Yarra Valley, Vic	
S-J. Bell	Managing *Botrytis* in the vineyard		
P.J. Costello, E.J. Bartowsky, P.A. Henschke	Strategies for successful induction of malolactic fermentation		
R.G. Dambergs	HACCP: what is it? do you need it? how do you do it ?- cheaply!		
	Quality Management Systems (ISO 9000)		
I.L. Francis	Oxidation of bottled wine: the role of ascorbic acid, and bottle storage conditions/non destructive measurement of white wine colour		
J.M. Eglinton and P.A. Henschke	Winemaking with alternative yeasts: influence on wine composition and flavour		
P.W. Godden	*Dekkera/Brettanomyces*, and the production of 4-ethylphenol during winemaking		
S-J. Bell	Agrochemical issues for grapegrowers and winemakers		
I.L. Francis	Tannins and mouth-feel: terminology and methods for sensory evaluation of red wines		
A.P. Pollnitz	Polyphenols, 'pigmented polymers' and red wine colour: results from the Tannin Project		
R.G. Dambergs	The use of NIR for the measurement of grape, juice and wine components		
A.P. Pollnitz	Cork taint – causes, detection and prevention	Roadshow seminar:	18 Sept 02
P.W. Godden	Managing stuck fermentation	Mornington, Vic	
S-J. Bell	The link between vineyard management and wine protein stability		
E.J. Bartowsky, D. Xia[3], G.H. Fleet[3], P.A. Henschke	Microbial induced oxidative spoilage of bottled red wine (acetic acid bacteria spoilage in wine)		
R.G. Dambergs	HACCP: what is it? Do you need it? How do you do it? – cheaply!		
	Quality Management Systems (ISO 9000)		
S-J. Bell	The fate of agrochemical residues during the winemaking process		
J.M. Eglinton and P.A. Henschke	Winemaking with alternative yeasts: influence on wine composition and flavour		
P.W. Godden	*Dekkera/Brettanomyces*, and the production of 4-ethylphenol during winemaking		
I.L. Francis	Tannins and mouth-feel: terminology and methods for sensory evaluation of red wines		
A.P. Pollnitz	Measuring desirable oak wood components in wine		
R.G. Dambergs	Grape colour measurement, G-G and NIR update		
I.L. Francis	Identification and measurement of key wine flavour compounds		
S.L. Brown	*Saccharomyces cerevisiae* mannoproteins which reduce visible haze in white wine	DHVO Research Seminar Plant Research Centre, Urrbrae, SA	25 Sept 02
A.D. Coulter	*Dekkera/Brettanomyces*, and the production of 4-ethylphenol during winemaking	Presentation and tasting provided to Judges at the Royal Adelaide Wine Show, Wayville, SA	30 Sept 02
I.L. Francis	The effects of tannins, anthocyanins, ethanol, and polysaccharides on red wine mouth-feel	ASVO Symposium – *The use of gases*, Burnside, SA	10 Oct 02
C.S. Stockley	Moderation in Australia – policy and achievements	Vinsalud Chile 2002 International Wine and Health Conference in Santiago, Chile	20-23 Oct 02
I.L. Francis	Grape quality measures	Victoria and Murray Valley Winegrape Growers Council, Merbein Wine Grapegrowers group, Mildura TAFE, Vic	12 Nov 02
P.W. Godden	Update on the AWRI trial of the technical performance of wine closures	Roadshow seminars:	18 Nov 02
	Dekkera/Brettanomyces, and the production of 4-ethylphenol during winemaking	Swan Valley, WA	
M.A. Sefton	Cork taint – causes, detection and prevention		
	Oxidation of bottled wine: the role of ascorbic acid, and bottle storage conditions/non destructive measurement of white wine colour		
S-J. Bell	The link between vineyard management and wine protein stability		
	Agrochemical issues for grapegrowers and winemakers		
P.A. Henschke	Gene technology: introductory principles and improvement of grape and wine production		
	Microbial induced oxidative spoilage of bottled red wine (acetic acid bacteria spoilage in wine)		
R.G. Dambergs	HACCP: what is it? Do you need it? How do you do it? – cheaply!		
	Grape colour measurement and NIR		
M.J. Herderich	Polyphenols, 'pigmented polymers' and red wine colour: results from the Tannin Project		
	Tannins and mouth-feel: terminology and methods for sensory evaluation of red wines (mouth-feel wheel, and the results of some recent tannin sensory studies)		

Staff	Title of talk	Where and to whom	Date
P.W. Godden	Update on the AWRI trial of the technical performance of wine closures *Dekkera/Brettanomyces,* and the production of 4-ethylphenol during winemaking	Roadshow seminars: Great Southern WA	20 Nov 02
M.A. Sefton	Cork taint – causes, detection and prevention Oxidation of bottled wine: the role of ascorbic acid, and bottle storage conditions/non destructive measurement of white wine colour		
S-J. Bell	The link between vineyard management and wine protein stability Agrochemical issues for grapegrowers and winemakers		
P.A. Henschke	Strategies for successful induction of malolactic fermentation Microbial induced oxidative spoilage of bottled red wine (acetic acid bacteria spoilage in wine)		
R.G. Dambergs	HACCP: what is it? Do you need it? How do you do it? – cheaply! Grape colour measurement and NIR		
M.J. Herderich	Polyphenols, 'pigmented polymers' and red wine colour: results from the Tannin Project Tannins and mouth-feel: terminology and methods for sensory evaluation of red wines (mouth-feel wheel, and the results of some recent tannin sensory studies)		
C.S. Stockley	The changing face of moderation in Australia	2002 APSAD Alcohol and Drug Conference, Adelaide, SA	20 Nov 02
P.W. Godden	Update on the AWRI trial of the technical performance of wine closures *Dekkera/Brettanomyces*, and the production of 4-ethylphenol during winemaking	Roadshow seminars: Margaret River, WA	22 Nov 02
M.A. Sefton	Cork taint – causes, detection and prevention Oxidation of bottled wine: the role of ascorbic acid, and bottle storage conditions/non destructive measurement of white wine colour		
S-J. Bell	The link between vineyard management and wine protein stability Salty wine Agrochemical issues for grapegrowers and winemakers		
R.G. Dambergs	HACCP: what is it? Do you need it? How do you do it? – cheaply! Grape colour measurement and NIR		
P.A. Henschke	Gene technology: introductory principles and improvement of grape and wine production		
M.J. Herderich	Polyphenols, 'pigmented polymers' and red wine colour: results from the Tannin Project Tannins and mouth-feel: terminology and methods for sensory evaluation of red wines (mouth-feel wheel, and the results of some recent tannin sensory studies)		
I.L. Francis	Colour measurements – the basics	Interwinery Analysis Group, Interwinery Group Review Seminar, RACV Country Club, Healesville Vic	22 Nov 02
S.L. Brown, M.A. de Barros Lopes, P.B. Høj, E.J. Waters	Investigations into the mechanism of action and biological role of *S. cerevisiae* mannoproteins which reduce visible haziness in white wine	2nd Yeast Products and Discovery meeting, Melbourne, Vic	27-29 Nov 02
K.S. Howell, E.J. Bartowsky, G.H. Fleet[3], P.A. Henschke	Yeast strain dynamics during mixed culture wine fermentation and effect on wine composition		
S. Dillon, E.J. Bartowsky, P.B. Høj, L. Dulau[5], P.A. Henschke	Can yeast strain affect colour and phenolic content of Shiraz wine?		
A. Heinrich, J. Lahnstein[1], K. Ferguson[6], G. Currie[6], V. Jiranek[1], M. de Barros Lopes	Comparative proteomics: identifying novel proteins in the winemaking strain *Saccharomyces cerevisiae*		
J.M. Eglinton, A.J. Heinrich, A. Pollnitz, P.A. Henschke, M.A. de Barros Lopes	Using metabolomics to investigate cellular metabolic changes in an overproducing strain of *Saccharomyces cerevisiae*		
J.R. Bellon, J.M. Eglinton, A.P. Pollnitz, C. Hillier[7], M.A. de Barros Lopes	Hybrid wine yeasts with unique fermentation characteristics		
I.L. Francis	Characterising specific astringency properties of red wines	Australasian Association for Chemosensory Science 5th Scientific meeting, Heron Island Qld	11 Dec 02

Staff	Title of talk	Where and to whom	Date
P.A. Henschke	Sulfide biochemistry: microbiological origin and control of sulfur-compounds in winemaking	Winemakers from Orlando Wyndham, Rowland Flat, SA	28 Jan 03
P.W. Godden	*Dekkera/Brettanomyces* and the production of negative sensory compounds during winemaking (tasting and presentation)	Beringer Blass staff at Saltram winery, Barossa Valley, SA	31 Jan 03
M.A. Gishen, P.G. Iland[1,8], R.G. Dambergs, D. Cozzolino, I.L. Francis, P.B. Høj	Grape and wine quality-attributes and measurements	Victorian Wine Industry Association's *Grape quality and industry outlook* conference Victoria University Convention Centre, Sunshine, Vic	30–31 Jan 03
P.W. Godden	*Dekkera/Brettanomyces* and the production of negative sensory compounds during winemaking (tasting and presentation)	Winemakers at Beringer Blass Wines	31 Jan 03
Y. Hayasaka, R. Asenstorfer, A.E. Håkansson, M.J. Herderich	Screening for potential wine pigments in red wine using tandem mass spectrometry	The 19th conference of Australian and New Zealand Society for Mass Spectrometry held in Lorne, Victoria	2-6 Feb 03
P.W. Godden	AWRI industry support and development activities – the role of the Industry Services team	A group of Thai winemakers, Urrbrae, SA	17 Mar 03
P.A. Henschke	Overview of wine microbiology/biotechnology projects		
R.E. Day[2], P.J. Dawson[9], P.B. Høj, I.S. Pretorius	Response to WFA R&D Review	WFA R&D Review Committee, National Wine Centre, Adelaide SA	21 Mar 03
M.J. Herderich	Yeast-mediated formation of anthocyanin-derived pigments in red wine	*Uncovering the mysteries of red wine pigments* American Chemical Society, New Orleans, USA	23 Mar 03
I.S. Pretorius	A general introduction to The Australian Wine Research Institute	E&J Gallo, US senior production team, AWRI, Urrbrae, SA	26 Mar 03
G.K. Skouroumounis, M.J. Kwiatkowski, I.L. Francis, M.A. Sefton, E.J. Waters	Oxidation of bottled wine: non-destructive measurement of white wine colour, the role of ascorbic acid, and bottle storage conditions		
M.A. Sefton	Measuring aroma compounds in wine		
I.L. Francis	Winegrape tannin and colour specification		
P.A. Henschke	Wine microbiology/biotechnology projects: microbial modulation of wine sensory properties		
P.W. Godden	The role of the AWRI Industry Services team		
M.J. Herderich	Potential cardiovascular benefits of moderate wine consumption	*Grand rounds in clinical pharmacology* Vanderbilt University Medical Centre, Nashville, USA	1 Apr 03
P.B. Høj, I.S. Pretorius, R.E. Day[2]	Beyond the idea: the wine industry's approach to bridging the gap between the idea and its transformation to commercial outcomes The importance of industry/researcher communication as a driver for a 'can-do' culture in pursuit of excellence	*Innovation: beyond the idea* seminar The Warren Centre, Powerhouse Museum, Ultimo, NSW	3 Apr 03
M.J. Herderich	Yeast mediated formation of anthocyanin-derived pigments in red wine	University of Auckland, New Zealand	4 Apr 03
I.L. Francis	Common wine faults and how to avoid them	Blackwood Winemakers and Brewers Club, Blackwood, SA	16 Apr 03
M. Gishen, R.G. Dambergs, L. Janik, W.U. Cynkar, D. Boehm, I.L Francis, D. Cozzolino and P.B. Høj	NIR in the wine industry	Wine users group seminar at FOSS Directions 2003 conference, Taupo, New Zealand	28–29 May 03
M. Gishen and G. Harvey[10]	Legal metrology in the wine industry		
L. Janik, D. Buick, M. Holdstock, A. Massis, A. Catalano, M. Henry[11], M. Gishen	FOSS 'WineScan'—calibration development for wine analysis		
R.G. Dambergs	Measuring grape colour	CRCV Field Seminar, Mildura, Vic	28 May 03

Staff	Title of talk	Where and to whom	Date
P.A. Henschke M.A. de Barros Lopes P.B. Høj M.A. Sefton I.L. Francis	Fermentation technologies Metabolic profiling Wine flavour and aroma. What can we measure now? Flavour compounds in wine and grapes Sensory analysis	*First workshop on wine flavour and aroma from vine to palate.* CSIRO Plant Industry, Waite Campus, Urrbrae SA	30 May 03
P.A. Henschke	Present and future developments in alcoholic fermentation management in Australia	Formazione Permanente Vitivinicola Conference Conegliano, Italy	12-13 Jun 03
I.S. Pretorius	Novel approaches to winemaking – tailoring wine yeast for future challenges	1st FEMS Congress of European Microbiologists, Ljubijana, Slovenia	29 Jun to 3 Jul 03

Workshops

Staff	Title of presentation	Location	Date
P.W. Godden A.D. Coulter, E.M.C. Robinson, M. Gishen, P.W. Godden M. Gishen E.M.C. Robinson A.D. Coulter	Trouble free winemaking – causes and prevention of common wine instabilities Tasting of simulated faulty wines Laboratory analysis and quality management Introduction to AWRI technical manual Website Isolation of hazes/deposits and use of microscope What bug is that?	Roadshow workshop presented to winemakers and grapegrowers, Coonawarra, SA, *Trouble free winemaking and troubleshooting wine instability problems*	3 Jul 02
P.W. Godden A.D. Coulter, E.M.C. Robinson, M. Gishen, P.W. Godden Mark Gishen E.M.C. Robinson A.D. Coulter	Trouble free winemaking – causes and prevention of common wine instabilities Tasting of simulated faulty wines Laboratory analysis and quality management Introduction to AWRI technical manual Website Isolation of hazes/deposits and use of microscope What bug is that?	Roadshow workshop presented to winemakers and grapegrowers, Hobart, Tas, *Trouble free winemaking and troubleshooting wine instability problems*	16 Aug 02
R.G. Dambergs	NIRS in the wine industry Quality Management Systems – ISO 9002	Grapegrowers and winemakers in Swan Valley, Great Southern and Margaret River, WA	18-22 Aug 02
S-J. Bell	*Research to Practice*™ Grapevine Nutrition	Langhorne Creek, SA Mildura, Vic	4-5 Sept 02 24-25 Sept 02
R.G. Dambergs	NIRS in the wine industry Quality Management Systems – ISO 9002	Grapegrowers and Winemakers in Yarra Valley and Mornington Peninsula, Vic	16-18 Sept 02
S-J. Bell	*Research to Practice*™ Grapevine Nutrition	Naracoorte, SA	17-18 Oct 02
P.W. Godden A.D. Coulter, E.M.C. Robinson, M. Gishen, P.W. Godden M. Gishen E.M.C. Robinson A.D. Coulter	Trouble free winemaking – causes and prevention of common wine instabilities Tasting of simulated faulty wines Laboratory analysis and quality management Introduction to AWRI technical manual Website Isolation of hazes/deposits and use of microscope What bug is that?	La Trobe University's Bundoora Campus, members of the Victorian wine industry	29, 30, 31 Oct 02
C.S. Stockley	Workshop to summarise GWRDC-funded research outcomes for wine and health Potential cardioprotective activities of wine components based on synergistic interaction with vitamin E	The Australian Wine Research Institute, Urrbrae, SA	13 Dec 02
S-J. Bell	*Research to Practice* Winegrape Quality Management workshop	Great Southern Swan Valley, WA	6-7 Jan 03 9-10 Jan 03

Posters presented

Author	Title of poster	Organisation and location	Date
K.S. Howell, E.J. Bartowsky, G.H. Fleet, P.A. Henschke	Yeast strain dynamics during mixed culture wine fermentation and effect of wine composition	International Union of Microbiological Societies, Paris, France	27 Jul – 1 Aug 02
S.L. Brown, M.A. de Barros Lopes, P.B. Høj, E.J. Waters	Investigation into the mechanism of action and biological role of *S. cerevisiae* mannoproteins that reduce visible haziness in wine	2002 Yeast Genetics and Molecular Biology Meeting, Madison, Wisconsin, USA	30 Jul-4 Aug 02
E.J. Bartowsky, J.M. McCarthy, I.L. Francis, P.A. Henschke	Assessment of importance of bacterial strain on diacetyl production in red and white wine and its sensory contribution	Seventh Symposium on Lactic Acid Bacteria: *Genetics, Metabolism and Applications*, Egmond aan Zee, The Netherlands	1-5 Sept 02
J.E. Eglinton, A.J. Heinrich, A. Pollnitz, P.A. Henschke, M.A. de Barros Lopes	Using metabolomics to investigate cellular metabolic changes in an overproducing strain of *Saccharomyces cerevisiae*	2nd Australian Conference on Yeast: *Products and Discovery*, Melbourne, Vic	27-29 Nov 02
J.E. Eglinton, P.A. Henschke	*Saccharomyces bayanus* – an alternative wine yeast?		
K.S. Howell, E.J. Bartowsky, G.H. Fleet, P.A. Henschke	Yeast strain dynamics during mixed culture wine fermentations and effect on wine composition		
S. Dillon, E.J. Bartowsky, P.B. Høj, L. Dulau[5], P.A. Henschke	Can yeast strain affect colour and phenolic content of Shiraz wine?		
D. Torrea, C. Ancin[12]	Biogenic amines in wines obtained with different yeast strains: relationship with their precursor amino acids		
D. Torrea, C. Ancin[12]	Production of volatile metabolites during fermentation of grape must inoculated with two different nitrogen requiring yeast strains		
R.G. Dambergs, D. Cozzolino, W.U. Cynkar, M.B. Esler, L.J. Janik, I.L. Francis, M. Gishen	Strategies to minimise matrix-related error with NIRS analysis of wine grape quality parameters	11th International NIRS Conference, Spain	Apr 03
D. Cozzolino, M.J. Kwiatkowski, M. Parker, M. Gishen, R.G. Dambergs, W.U. Cynkar, M.J. Herderich	Measurement of phenolic compounds during red wine fermentation by near infrared transmission (NIT) spectroscopy		
D. Cozzolino, M. Gishen, R.G. Dambergs, W.U. Cynkar	Determination of colour and pH of red grapes using a diode array instrument		
D. Cozzolino, M. Gishen, R.G. Dambergs, W.U. Cynkar, I.L. Francis	Calibration transfer between different NIR instruments for determination of colour in red grapes		
M.A. Daniel	The consumption of ß-damascenone in wine	The 19th Royal Australian Chemical Institute Organic Conference (19RACI-OC), Cumberland Conference Resort, Lorne Victoria	6-11 Jul 03
J.M. Eglinton, A.J. Heinrich, A.P. Pollnitz, P.A. Henschke, M.A. de Barros Lopes	Investigating the link between yeast genes and wine flavour	21st International Conference on Yeast Genetics and Molecular Biology (ICGMB) Göteborg, Sweden	7-12 Jul 03
I.S. Pretorius	Rme1p induces FLO11 expression through an 11 bp Rme1p response element		
	Ras regulates the carnitine shuttle in yeast		
	Maltotriose transport in yeast		
	Factors affecting fructose consumption in wine yeast		
	Influence of flocculation on ethanol production by recombinant yeast		

Other activities

Staff	Activity	Date
P.W. Godden, E.M.C. Robinson, M. Gishen, T.A. Weber, A.D. Coulter, I.L. Francis, K.A. Lattey	Advanced Wine Assessment Course, Waite Campus, Urrbrae SA	23-26 Jul 02
P.W. Godden	South Australian Vine Improvement, Riesling clonal selection trial. Participated as a panel member during a public tasting	21 Feb 03

1 School of Agriculture and Wine, The University of Adelaide
2 Pernod Ricard
3 Department of Food Science, University of New South Wales
4 Field Consulting Services
5 Lallemand
6 School of Botany, University of Melbourne
7 Flinders University
8 now, Davidson Viticulture
9 Hardy Wine Company
10 National Standards Commission
11 FOSS Pacific
12 Universidad Publica de Navarra

APPENDIX 2. Teaching responsibilities of Institute staff during 2002/03

Subject	No. of lectures	Institute staff
2002 – Semester 2		
The University of Adelaide		
3045WT Advances in oenology	3	E.J. Bartowsky
	1	M.A. de Barros Lopes
	2	I.L. Francis
	1	P.W. Godden
	4	P.A. Henschke
	5	M.J. Herderich
	1	P.B. Høj
3003WT Wine packaging and quality management	1	P.W. Godden
2022WT Sensory studies	2	I.L. Francis
Flinders University		
MMED 3921 Industrial and pharmaceutical microbiology	1	P.A. Henschke
2003 – Semester 1		
The University of Adelaide		
1958 Wine packaging and quality management	1	M. Gishen
2001WT for undergraduates and 7030WT for postgraduates (final year wine marketing students as part of the course entitled Wine and Society)	2	C.S. Stockley
3005WT Grape industry practice policy and communication	Approx. 50 hours	C.S. Stockley and P.B. Høj
2580 Stabilisation and clarification	3	E.J. Waters
2580 Stabilisation and clarification	1	A.D. Coulter
1005/3113 Winemaking	2	P.A. Henschke
3009WT Advanced sensory practice	8 hours	P.W. Godden
3009WT Advanced sensory practice	2	I.L. Francis
3002WT Biotechnology in the Food and Wine Industry	1	M.A. de Barros Lopes
LaTrobe University		
Australian wine industry and structure	1 hour	P.W. Godden
Defining and measuring grape and wine quality	1 hour	P.W. Godden
Trouble free winemaking – the identification and management of common wine instabilities	1.5 hours	P.W. Godden
Dekkera/Brettanomyces, and the production of negative sensory compounds during winemaking, including a tasting of wines containing key spoilage compounds	2 hours	P.W. Godden
An evaluation of the technical performance of various types of wine closure - *Results to 42 months post bottling*	1.5 hours	P.W. Godden

APPENDIX 3. Graduate and Honours student supervision responsibilities of Institute staff for 2002/03

Student	Supervisor/s	Source of funds
PhD		
S.L. Brown	E.J. Waters, M.A. de Barros Lopes, P.B. Høj	GWRDC
F. Carrau	P.A. Henschke, E. Dellacassa[1]	University of the Republic of Uruguay staff member
M.A. Daniel	G.M. Elsey, M.A. Sefton, M. Perkins[2]	CRCV
M. de Sa	M.J. Herderich, I.L. Francis, A.P. Pollnitz	CRCV
J.M. Eglinton	P.A. Henschke, P. Langridge[3]	Institute staff
J. Gardner	V. Jiranek[3], M.A. de Barros Lopes	School of Agriculture and Wine – The University of Adelaide
W. Greenrod	M. Fenech[4], M. Abbey[4], P. Burcham[5], C.S. Stockley	GWRDC
A. Grimaldi	V. Jiranek[3], E.J. Bartowsky	School of Agriculture and Wine – The University of Adelaide /GWRDC
C. Guirado	G.K. Skouroumounis, E.J. Waters	Visiting French Postgraduate student (until 25 October 2002)
A.J. Heinrich	M.A. de Barros Lopes, V. Jiranek[3]	CRCV
K.S. Howell	P.A. Henschke, E.J. Bartowsky, G. Fleet[6], M.A. de Barros Lopes	University of NSW/GWRDC
A. Janusz	G.M. Elsey, M.A. Sefton, M. Perkins[2]	CRCV
C. McBryde	V. Jiranek[3], M.A. de Barros Lopes	School of Agriculture and Wine – The University of Adelaide
O.J. Macintyre	C. Colby[7], B. O'Neill[7], E.J. Waters, I.S Pretorius	School of Chemical Engineering – The University of Adelaide/GWRDC
R. Muhlack	C. Colby[7], B. O'Neill[7], E.J. Waters, P.B. Høj, A. Lim[8]	School of Chemical Engineering – The University of Adelaide/Hardy Wine Company/GWRDC
C.J. Puglisi	G.M. Elsey, M.A. Sefton, R. Prager[2]	CRCV
R. Ristic	P.J. Iland[3],[9], I.L. Francis, M.J. Herderich	GWRDC
H.E. Smyth	I.L. Francis, M.A. Sefton, M.J. Herderich	GWRDC
K.L. Wilkinson	G.M. Elsey, M.A. Sefton, R. Prager[2]	GWRDC
Masters		
M. Astorga	M.A. de Barros Lopes, V. Jiranek[3]	School of Agriculture and Wine, The University of Adelaide
D. Coates	E.J. Bartowsky	School of Agriculture and Wine, The University of Adelaide
Honours		
R.C. Brown	G.M. Elsey, D. Taylor[3]	Flinders University
M. Caldersmith	A.P. Pollnitz, S.J. Clarke[3]	School of Agriculture and Wine – The University of Adelaide
J.S. Crossman	G.M. Elsey	Flinders University
J. Guzinski	M.A. de Barros Lopes, Carolyn Leach[10]	Department of Genetics – The University of Adelaide
N.R. Sleep	G.M. Elsey, D.L. Capone	Flinders University
Industry placement		
B. Winstone,	M.A. de Barros Lopes	Department of Biotechnology, Flinders University Jan 03

Theses completed - Honours/PhD

Student	Hon/PhD	Title of thesis	Supervisors
J. Cartwright	Hons	The effect of interspecific *Saccharomyces* hybrids on the sensory and chemical profiles of wine	M.A. de Barros Lopes, A.J. Yap[3]
M. Fettke	Hons	The role of amino acids in wine yeast and lactic acid bacteria compatibility	P.A. Henschke, E.J. Bartowsky, P.J. Costello
K. Poole	PhD	Increasing the fermentation reliability of wine yeasts by enabling proline utilisation	M.A. de Barros Lopes, V. Jiranek[3]
K. van Leeuwen	Hons	The development of stable isotope dilution assays for the quantification of the important aroma compounds citronellol, 4-vinylguaiacol and 4-vinylphenol in wines	G.M. Esley

1 University of Republic of Uruguay
2 Flinders University
3 The University of Adelaide School of Agriculture and Wine
4 CSIRO Health Sciences and Nutrition
5 The University of Adelaide Department of Clinical and Experimental Pharmacology
6 University of New South Wales
7 The University of Adelaide School of Chemical Engineering
8 Hardy Wine Company
9 now, Davidson Viticulture
10 The University of Adelaide Department of Genetics

653a	Francis, L.; Cynkar, W. Investigation of an 'electronic nose'. Aust. NZ Wine Ind. J. 15: 54–56; 2000.
694	Walker, R.; Blackmore, D.; Clingeleffer, P.; Godden, P.; Francis, L.; Valente, P.; Robinson, E. The effects of salinity on vines and wines. Aust. Vitic. 6(4): 11–14, 16–18, 20–22; 2002.
695	Day, R.E.; Gishen, M.; Godden, P.W.; Hughes, J.B. Producing unique wine styles for overseas markets. Blair, R.J.; Williams, P.; Høj, P.B., eds. Proceedings of the eleventh Australian wine industry technical conference; 7–11 October 2001; Adelaide, SA. Adelaide: Australian Wine Industry Technical Conference Inc.; 2002: 27–30.
696	Godden, P.W.; Francis, I.L.; Field, J.B.F.; Gishen, M.; Coulter, A.D.; Valente, P.J.; Høj, P.B.; Robinson, E.M.C. An evaluation of the technical performance of wine bottle closures. Blair, R.J.; Williams, P.; Høj, P.B., eds. Proceedings of the eleventh Australian wine industry technical conference; 7–11 October 2001; Adelaide, SA. Adelaide: Australian Wine Industry Technical Conference Inc.; 2002: 44–52.
697	Francis, I.L.; Gawel, R.; Iland, P.G.; Vidal, S.; Cheynier, V.; Guyot, S.; Kwiatkowski, M.J.; Waters, E.J. Characterising mouth-feel properties of red wines. Blair, R.J.; Williams, P.; Høj, P.B., eds. Proceedings of the eleventh Australian wine industry technical conference; 7–11 October 2001; Adelaide, SA. Adelaide: Australian Wine Industry Technical Conference Inc.; 2002: 123–127.
698	Ristic, R.; Francis, I.L.; Gawel, R.; Iland, P.G. Relationship between seed composition and grape and wine quality. Blair, R.J.; Williams, P.; Høj, P.B., eds. Proceedings of the eleventh Australian wine industry technical conference; 7–11 October 2001; Adelaide, SA. Adelaide: Australian Wine Industry Technical Conference Inc.; 2002: 145–149.
699	Kwiatkowski, M.J.; Peng Z.K.; Waters, E.J.; Godden, P.W.; Day, C.J.; Clarke, S.J.; Herderich, M.J. Polyphenols, pigmented polymers and red wine colour: results of the 2001 large scale winemaking trial. Blair, R.J.; Williams, P.; Høj, P.B., eds. Proceedings of the eleventh Australian wine industry technical conference; 7–11 October 2001; Adelaide, SA. Adelaide: Australian Wine Industry Technical Conference Inc.; 2002: 150–151.
700	Eglinton, J.M.; Bellon, J.R.; Pollnitz, A.P.; Heinrich, A.J.; Yap, A.J.; Langridge, P; Henschke, P.A.; Høj, P.B.; de Barros Lopes, M.A. Genetic approaches to the improvement of wine. Blair, R.J.; Williams, P.; Høj, P.B., eds. Proceedings of the eleventh Australian wine industry technical conference; 7–11 October 2001; Adelaide, SA. Adelaide: Australian Wine Industry Technical Conference Inc.; 2002: 152–156.
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APPENDIX 5. Institute Committees

Staff member	Management Advisory	Research Steering	Industry Services Steering	Communication Steering	Analytical Service Steering	Information Technology	Biosafety	Occupational Health and Safety	Staff Code Negotiation
Eveline Bartowsky						X		X	X
Sally-Jean Bell	X	X	X	X	X				
Rae Blair	X			C					
Amanda Cook								X	
Adrian Coulter	X		X		X				
Catherine Daniel				X					
Miguel de Barros Lopes	X	X					X		
Maria de Sa								X	
Rachel Edwards						X			
Jeff Eglinton				X		C	C		
Peter Eichinger	X		X		X				
Leigh Francis	X	X	X						
Mark Gishen	X	X	X		C				
Holger Gockowiak							X		
Peter Godden	X	X	C	X	X				
Yoji Hayasaka	X	X	X						
Paul Henschke	X	X			X			C	
Markus Herderich	X	X							
Peter Høj	C	X	X	X	X	X			X
Matthew Holdstock							X		
Hans Muhlack	X								X
Ingrid Oats									X
Ken Pocock							X	X	
Alan Pollnitz						X			
Sakkie Pretorius	X	C							
Mark Sefton	X	X			X				
Creina Stockley				X					
Randell Taylor						X			
Elizabeth Waters	X	X							

C = denotes holder of Chair

Front row

Aggie Janusz, Julia Crossman, Rachel Brown, Nicola Sleep, Maria Mills, Heather Brooks, Creina Stockley, Narelle D'Costa, Amanda Cook, Heather Donnell

Second row

Jelena Jovanovic, Yoji Hayasaka, Sandra Lloyd-Davies, Heather Smyth, Kate Lattey, Kerry Wilkinson, Hans Muhlack, Merran Daniel, Gayle Baldock, George Skouroumounis, Jenny Bellon, Lorelie Flood, Mark Gishen, Anna Catalano, Rachel Edwards, Athina Massis, Rhonda Packer, Pauline Jorgensen, Melissa Francis, Rae Blair

Third row

Peter Høj, Randell Taylor, Miguel de Barros Lopes, Anthony Heinrich, Diego Torrea, Adrian Coulter, Jaro Guzinski, Holger Gockowiak, Paul Henschke, Les Janik, Jean McIntyre, Wies Cynkar, Maria de Sa, Catherine Daniel, Simon Dillon, Julie McConnell, Daniel Cozzolino, Dimitra Capone, Bob Dambergs, Elizabeth Waters, Kevin Pardon, Richard Muhlack, Peter Eichinger, Mariola Kwitakowski, Sakkie Pretorius

Fourth row

Ingrid Oats, Paul Smith, Markus Herderich, Leigh Francis, Patrik Jones, Eveline Bartowsky, Alan Pollnitz, Tracey Siebert, Ken Pocock, Sally-Jean Bell, Ella Robinson, Gordon Elsey, David Boehm, Geoff Cowey, Mark Sefton, Matthew Cream, Greg Ruediger, Peter Godden

Absent

Kate Beames, Belinda Bramley, Jeff Eglinton, Matthew Holdstock, Kate Howell, Danielle Leedham, Jane McCarthy, Emma-Kate White

The staff of The Australian Wine Research Institute